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(54) Title: METHOD AND REAGENT FOR TREATMENT OF ARTHRITIC CONDITIONS, INDUCTION OF GRAFT TOLERANCE AND REVERSAL OF IMMUNE RESPONSES			
(57) Abstract An enzymatic nucleic acid molecule which cleaves RNA associated with development or maintenance of an arthritic condition, induction of graft tolerance or reversal of an immune response. In particular, the ribozyme sequences are directed to an mRNA encoding B7-1, B7-2, B7-3, CD40 and/or stromelysin. Also provided are ribozymes where the uracil in positions 4 and/or 7 are substituted, as well as methods for the synthesis of 2'-alkylnucleotides, 2'-O-alkylthioalkyl, or 2'-alkylthioalkylnucleotides. The application further describes a method for diprotection of RNA with aqueous ethylamine, a method for synthesis of a basic ribonucleoside mimetics, and transcription units comprising an RNA polymerase II promoter, a U6 small nuclear promoter, or an adenovirus VA1 promoter system.			

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METHOD AND REAGENT FOR TREATMENT OF ARTHRITIC
CONDITIONS. INDUCTION OF GRAFT TOLERANCE AND
REVERSAL OF IMMUNE RESPONSES

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Background of the Invention

The following is a discussion of relevant art, none of which is admitted to be prior art to the present invention.

In one aspect, this invention relates to methods for inhibition of osteoarthritis, in particular, inhibition of genetic expression which leads to a reduction or elimination of extracellular matrix digestion by matrix metalloproteinases.

There are several types of arthritis, with osteoarthritis and rheumatoid arthritis being predominant. Osteoarthritis is a slowly progressive disease characterized by degeneration of articular cartilage with proliferation and remodeling of subchondral bone. It presents with a clinical picture of pain, deformity, and loss of joint motion. Rheumatoid arthritis is a chronic systemic inflammatory disease. Rheumatoid arthritis may be mild and relapsing or severe and progressive, leading to joint deformity and incapacitation.

Arthritis is the major contributor to functional impairment among the older population. It is the major cause of disability and accounts for a large proportion of the hospitalizations and health care expenditures of the elderly. Arthritis is estimated to be the principal cause of total incapacitation for about one million persons aged 55 and older and is thought to be an important contributing cause for about one million more.

Estimating the incidence of osteoarthritis is difficult for several reasons. First, osteoarthritis is diagnosed objectively on the basis of reading radiographs, but many people with radiologic evidence of disease have no obvious symptoms. Second, the estimates of prevalence are based upon clinical evaluations because radiographic data is not available for all afflicted joints. In the NHANESI survey of 1989, data were based upon a thorough musculoskeletal evaluation during which any abnormalities of the spine, knee,

hips, and peripheral joints were noted as well as other specific diagnoses. Based on these observations, 12% of the US population between 25 and 74 years of age have osteoarthritis.

It is generally agreed that rheumatoid arthritis has a world-wide distribution and affects all racial and ethnic groups. The exact prevalence in the US is unknown but has been estimated to range between 0.5% and 1.5%. Rheumatoid arthritis occurs at all age levels and generally increases in prevalence with advancing age. It is 2-3 times more prevalent in women than in men and peak incidence occurs between 40-60 years of age. In addition to immunological factors, environmental, occupational and psychosocial factors have been studied for potential etiologic roles in the disease.

The extracellular matrix of multicellular organisms plays an important role in the formation and maintenance of tissues. The meshwork of the extracellular matrix is deposited by resident cells and provides a framework for cell adhesion and migration, as well as a permeability barrier in cell-cell communication. Connective tissue turnover during normal growth and development or under pathological conditions is thought to be mediated by a family of neutral metalloproteinases, which are zinc-containing enzymes that require calcium for full activity. The regulation of metalloproteinase expression is cell-type specific and may vary among species.

The best characterized of the matrix metalloproteinases, interstitial collagenase (MMP-1), is specific for collagen types I, II, and III. MMP-1 cleaves all three chains of the triple helix at a single point initiating sequential breakdown of the interstitial collagens. Interstitial collagenase activity has been observed in rheumatoid synovial cells as well as in the synovial fluid of patients with inflammatory arthritis. Gelatinases (MMP-2) represent a subgroup of the metalloproteinases consisting of two distinct gene products; a 70 kDa gelatinase expressed by most connective tissue cells, and a 92 kDa gelatinase expressed by inflammatory phagocytes and tumor cells. The larger enzyme is expressed by macrophages, SV-40 transformed fibroblasts, and neutrophils. The smaller enzyme is secreted by H-ras transformed bronchial epithelial cells and tumor cells, as well as normal human skin fibroblasts. These enzymes degrade gelatin (denatured collagen) as well as native

collagen type XI. Stromelysin (MMP-3) has a wide spectrum of action on molecules composing the extracellular matrix. It digests proteoglycans, fibronectin, laminin, type IV and IX collagens and gelatin, and can remove the N-terminal propeptide region from procollagen, thus activating the collagenase. It has been found in human cartilage extracts, rheumatoid synovial cells, and in the synovium and chondrocytes of joints in rats with collagen-induced arthritis.

Both osteoarthritis and rheumatoid arthritis are treated mainly with compounds that inhibit cytokine or growth-factor induced synthesis of the matrix metalloproteinases which are involved in the extracellular matrix destruction observed in these diseases. Current clinical treatments rely upon dexamethasone and retinoid compounds, which are potent suppressors of a variety of metalloproteinases. The global effects of dexamethasone and retinoid treatment upon gene expression in treated cells make the development of alternative therapies desirable, especially for long term treatments. Recently, it was shown that gamma-interferon suppressed lipopolysaccharide induced collagenase and stromelysin production in cultured macrophages. Also, tissue growth factor- β (TGF- β) has been shown to block epidermal growth factor (EGF) induction of stromelysin synthesis in vitro. Experimental protocols involving gene therapy approaches include the controlled expression of the metalloproteinase inhibitors TIMP-1 and TIMP-2. Of the latter three approaches, only γ -interferon treatment is currently feasible in a clinical application.

Sullivan and Draper, International PCT Publication No. WO 94/02595 and Draper *et al.*, International PCT Publication No. WO 95/13380 disclose the use of ribozymes to treat arthritis.

In a second aspect, the invention relates to methods for the induction of graft tolerance, treatment of autoimmune diseases, inflammatory disorders and allergies in particular, by inhibition of B7-1, B7-2, B7-3 and CD40.

An adaptive immune response requires activation, clonal expansion, and differentiation of a class of cells termed T lymphocytes (T cells). T cell activation is a multi-step process requiring several signalling events between

the T cell and an antigen presenting cell. The ensuing discussion details signals that are exchanged between T cells and antigen presenting B cells. Similar pathways are thought to occur between T cells and other antigen presenting cells such as monocytes or follicular dendritic cells.

5 T cell activation is initiated when the T-cell receptor (TCR) binds to a specific antigen that is associated with the MHC proteins on the surface of an antigen presenting cell. This primary stimulus activates the T cell and induces expression of CD40 ligand (CD40L) on the surface of the T cell. CD40L then interacts with its cognate receptor, CD40, which is constitutively expressed on
10 the surface of B cells; CD40 transduces the signal leading to B cell activation. B cell activations result in the expression of B7-1, B7-2 and/or B7-3, which in turn interacts with constitutively expressed CD28 on the surface of T cells. The interaction generates a secondary co-stimulatory signal that is required to fully activate the T cell. Complete T cell activation via the T cell receptor and CD28
15 leads to cytokine secretion, clonal expansion, and differentiation. If the T cell receptor is engaged, absence of this secondary co-stimulus mediated by CD28, then the T cell is inactivated, either by clonal anergy (non-responsiveness or reduced reactivity of the immune system to specific antigen(s)) or clonal deletion (Jenkins et al., 1987 *Proc. Natl. Acad. Sci. USA* 84, 5409). Thus, engagement of the TCR without a concomitant costimulatory signal results in a state of tolerance toward the specific antigen recognized by the T cell. This co-stimulatory signal can be mediated by the binding of B7-1 or B7-2 or B7-3, present on activated antigen-presenting cells, to CD28, a receptor that is constitutively expressed on the surface of the T cell
20 (Marshall et al., 1993 *J Clin Immun* 13, 165-174; Linsley, et al., 1991 *J Exp Med* 173, 721; Koulouva et al., 1991 *J Exp Med* 173, 759; Harding et al., 1992 *Nature* 356, 607).

Several homologs of B7 (now known as B7-1; Cohen, 1993 *Science* 262, 844) are expressed in activated B cells (Freeman et al., 1993 *Science* 262, 907; Lenschow et al., 1993 *Proc Natl Acad Sci USA* 90, 11054; Azuma et al., 1993 *Nature* 366, 76; Hathcock et al., 1993 *Science* 262, 905; Freeman et al., 1993 *Science* 262, 909). B7-1 and B7-3 are only expressed on the surface of a subset of B cells after 48 hours of contact with T cells. In contrast, B7-2 mRNA is constitutively expressed by unstimulated B cells and increases 4-fold
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within 4 hours of activation (Freeman et al., 1993 *Science* 262, 909; Boussiotis et al., 1993 *Proc Natl Acad Sci USA* 90, 11059). Since T cells commit to either the anergy or the activation pathway within 12-24 hours of the initial TCR signal, it is thought that B7-2 is the molecule responsible for the primary
5 costimulatory signal. B7-1 and B7-3 may provide a subsequent signal necessary for clonal expansion. Antibodies to B7-2 completely block T cell proliferation in a mixed lymphocyte reaction (Azuma et al., 1993 *supra*), supporting the central role of B7-2 in T cell activation. These experiments indicate that inhibition of B7-2 expression (for example with a ribozyme) would
10 likely induce anergy. Similarly, inhibition of CD40 expression by a ribozyme would prevent B7-2 upregulation and could induce tolerance to specific antigens.

B7 (B7-1) is a 60 KD modified trans-membrane glycoprotein usually present on the surface of antigen presenting cells (APC). B7 has two ligands—
15 CD28 and CTLA4. Interaction of B7-1 with CD28 and/or CTLA4 causes activation of T cell responses (Janeway and Bottomly, 1994 *Cell* 76, 275).

B7-2 is a 70 KD (34 KD unmodified) trans-membrane glycoprotein found on the surface of APCs. B7-2 encodes a 323 amino-acid protein which is 26 % identical to human B7-1 protein. Like B7-1, CD28 and CTLA4 are
20 selectively bound by B7-2. B7-2, unlike B7-1, is expressed on the surface of unstimulated B cells (Freeman et al., 1993 *supra*).

CD40 is a 45-50 KD surface glycoprotein found on the surface of late pre-B cells in bone marrow, mature B cells, bone marrow-derived dendritic cells and follicular dendritic cells (Clark and Ledbetter, 1994 *Nature* 367, 425).

25 Successful organ transplantation currently requires suppression of the recipient's immune system in order to prevent graft rejection and maintain good graft function. The available therapies, including cyclosporin A, FK506 and various monoclonal antibodies, all have serious side effects (Caine, 1992 *Transplantation Proceedings* 24, 1260; Fuleihan et al., 1994 *J. Clin. Invest.* 93,
30 1315; Van Gool et al., 1994 *Blood* 83, 176). In addition, existing therapies result in general immune suppression, leaving the patient susceptible to a variety of opportunistic infections. The ability to induce a state of long-term,

antigen-specific tolerance to the donor tissue would revolutionize the field of organ and tissue transplantation. Since organ graft rejection is mediated by T cell effector function, the goal is to block specifically the activation of the subset of T cells that recognize donor antigens. A limitation in the field of transplantation is the supply of donor organs (Nowak 1994 *Science* 266, 1148). The ability to induce donor-specific tolerance would substantially increase the chances of successful allografts, xenografts, thereby greatly increasing the donor pool.

Such transplantation includes grafting of tissues and/or organ ie., implantation or transplantation of tissue and/or organs, from the body of an individual to a different place within the same or different individual. Transplantation also involve grafting of tissues and/or organs from one area of the body to another. Transplantation of tissues and/or organs between genetically dissimilar animals of the same species is termed as allogeneic transplantation. Transplantation of animal organs into humans is termed xenotransplants (for a review see Nowak, 1994 *Science* 266, 1148).

One therapy currently being developed that has similar potential to induce antigen-specific tolerance is treatment with a CTLA4-Ig fusion protein. "CTLA4" is a homologue of CD28 that binds B7-1 and B7-2 with high affinity. The engineered, soluble fusion protein, CTLA4-Ig, binds B7-1, thereby blocking its interaction with CD28. The results of CTLA4-Ig treatment in animal studies are mixed. CTLA4-Ig treatment significantly enhanced survival rates and ameliorated the symptoms of graft-versus host disease in a murine bone marrow transplant model (Blazer et al., 1994 *Blood* 83, 3815). CTLA4-Ig induced long-term (>110 days) donor-specific tolerance in pancreatic islet xenografts (Lenschow et al., 1992 *Science* 257, 789). Conversely, in another study CTLA4-Ig treatment delayed but did not ultimately prevent cardiac allograft rejection (Turka, et al., 1992 *Proc Natl Acad Sci U S A* 89, 11102). Mice immunized with sheep erythrocytes in the presence of CTLA4-Ig failed to mount a primary immune response (Linsley, et al., 1992 *Science* 257, 792). A secondary immunization did elicit some response, however, indicating incomplete tolerance. Interestingly, identical results were obtained when CTLA4-Ig was administered 2 days after primary immunization, leading the authors to conclude that CTLA4-Ig blocked amplification rather than initiation

of the immune response. Since CTLA4-Ig has been shown to dissociate more rapidly from B7-2 compared with B7-1, this may explain the failure to induce long term tolerance in this model (Linsley et al., 1994 *Immunity* 1, 793).

5 CTLA4:Ig has recently been shown to ameliorate symptoms of spontaneous autoimmune disease in lupus-prone mice (Finck et al., 1994 *Science* 265, 1225).

Linsley et al., WO 92/00092 describe B7 antigen as a ligand for CD28 receptor on T cells. The application states that—

10 "The B7 antigen, or its fragments or derivatives are reacted with CD28 positive T cells to regulate T cell interactions with other cells..... B7 antigen or CD28 receptor may be used to inhibit interaction of cells associated with these molecules, thereby regulating T cell responses."

De Boer and Conroy, WO 94/01547 describe the use of anti-B7 and anti-CD40 antibodies to treat allograft transplant rejection, graft versus host disease and rheumatoid arthritis. The application states that—

15 "...anti-B7 and anti-CD40 antibodies...can be used to prevent or treat an antibody-mediated or immune system disease in a patient."

Since signalling via CD40 precedes induction of B-7, blocking the CD40-CD40L interaction would also have the potential to produce tolerance. According to one report, simultaneous treatment of mice with antibodies to
20 CD40L and sheep red blood cells produced antigen-specific tolerance for up to 3 weeks following cessation of treatment (Foy et al., 1993 *J Exp Med* 178, 1567). Anti-CD40L also produces antigen specific tolerance in a pancreatic islet transplant model (R. Noelle, personal communication). Targeted inhibition of CD40 expression in B cells in addition to B7 would therefore
25 afford double protection against activation of T cells.

Therapeutic agents used to prevent rejection of a transplanted organ are all cytotoxic compounds or antibodies designed to suppress the cell-mediated immune system. The side effects of these agents are those of immunosuppression and infections. The primary approved agents are
30 azathioprine, corticosteroids, cyclosporine; the antibodies are antilymphocyte or antithymocyte globulins. All of these are given to individuals who have been as closely matched as possible to their donors by both major and minor

histocompatibility typing. Since the principal problem in transplantation is an antigenic mismatch and the resulting need for cytotoxic therapy, any therapeutic improvement which decreases the local immune response without general immunosuppression should capture the transplant market.

- 5 Cyclosporine: At the end of the 1970's and early 1980's the introduction of cyclosporine revolutionized the transplantation field. It is a potent immunosuppressant which can inhibit immunocompetent lymphocytes specifically and reversibly. Its primary mechanism of action appears to be inhibition of the production and release of interleukin-2 by T helper cells. In addition it also interferes with the release of interleukin-1 by macrophages, as well as proliferation of B lymphocytes. It was approved by the FDA in 1983 and by 1989 was almost universally given to transplant recipients. At first it was believed that the toxicity and side effects from cyclosporine were minimal and it was hailed as a "wonder drug." Numerous side effects have been progressively cited, including the appearance of lymphomas, especially in the gastrointestinal tract; acute and chronic nephrotoxicity; hypertension; hepatotoxicity; hirsutism; anemia; neurotoxicity; endocrine and neurological complications; and gastrointestinal distress. It is now widely acknowledged that the non-specific side effects of the drug demand caution and close monitoring of its use. One-year survival rates for cadaver kidney transplants treated with cyclosporine is 80%, much better than the 50-60% rates without the drug. The one-year survival is almost 90% for transplants with related donors and the use of cyclosporine.

- 25 Azathioprine: In addition to cyclosporine, azathioprine is used for transplant patients. Azathioprine is one of the mercaptopurine class of drugs and inhibits nucleic acid synthesis. Patients are maintained indefinitely on daily doses of 1mg/kg or less, with a dosage adjusted in accordance with the white cell count. The drug may cause depression of bone marrow elements and may cause jaundice.

- 30 Corticosteroids: Prednisone, used in almost all transplant recipients, is usually given in association with azathioprine and cyclosporine. The dosage must be regulated carefully so as to prevent complications such as infection, development of cushingoid features, and hypertension. Usually the initial

maintenance prednisone dosage is 0.5 mg/kg/d. This dosage is usually further decreased in the outpatient clinic until maintenance levels of about 10 mg/d for adults are obtained. The exact site of action of corticosteroids on the immune response is not known.

- 5 Antithymoblast or antilymphocyte globulin (ALG) and antithymocyte globulin (ATG): These are important adjunctive immunosuppressants. They are effective, particularly in induction of immunosuppressive therapy and in the treatment of corticosteroid-resistant rejection. Both ALG and ATG can be made by immunizing horses, rabbits, or sheep; the main source is horses.
- 10 Lymphocytes from human peripheral blood, spleen, lymph nodes, or thymus serve as the immunogen.

- Tacrolimus: On April 13, 1994 the Food and Drug Administration approved another drug to help prevent the rejection of organ transplants. The drug, tacrolimus, was approved only for use in liver transplant patients. An
- 15 alternative to cyclosporine, the macrolide immunosuppressant tacrolimus is a powerful and selective anti-T-lymphocyte agent that was discovered in 1984. Tacrolimus, isolated from the fungus *Streptomyces tsukubaensis*, possesses immunodepressant properties similar to but more potent than cyclosporine. It inhibits both cell-mediated and humoral immune responses. Like
- 20 cyclosporine, tacrolimus demonstrates considerable interindividual variation in its pharmacokinetic profile. Most clinical studies with tacrolimus have neither been published in their entirety nor subjected to extensive peer review; there is also a paucity of published randomized investigations of tacrolimus vs. cyclosporine, particularly in renal transplantation. Despite these drawbacks,
- 25 tacrolimus has shown notable efficacy as a rescue or primary immunosuppressant therapy when combined with corticosteroids. The potential for reductional withdrawal of corticosteroid therapy with tacrolimus appears to be a distinct advantage compared with the cyclosporine. This benefit may be enhanced by reduced incidence of infectious complications,
- 30 hypertension and hypercholesterolemia reported by some investigators. In other respects, the tolerability profile of tacrolimus appears to be broadly similar to that of cyclosporine.

In addition to induction of graft tolerance, T cell anergy can be used to reverse autoimmune diseases. Autoimmune diseases represent a broad category of conditions. A few examples include insulin-dependent diabetes mellitus (IDDM), multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), myasthenia gravis (MG), and psoriasis. These seemingly disparate diseases all share the common feature of inappropriate immune response to specific self-antigens. Finck et al. *supra* have reported that CTLA4Ig treatment of mice blocked auto-antibody production in a mice model of SLE. In fact, this effect was observed even when the CTLA4Ig treatment was initiated during the advanced stages of the disease, suggesting that the autoimmune response was a reversible process.

Chappel, WO 94/11011 describes methods to treat autoimmune diseases by inducing tolerance to cells, tissues and organs. The application states that—

"Cells genetically engineered with DNA encoding a plurality of antigens of a cell, tissue, or organ to which tolerance is to be induced. The cells are free of co-stimulatory antigens, such as B7 antigen. Such cells induce T-cell anergy against the proteins encoded by the DNA, and may be administered to a patient in order to prevent the onset of or to treat an autoimmune disease, or to induce tolerance to a tissue or organ prior to transplantation."

Allergic reactions represent an immediate hypersensitivity response to environmental antigens, typically mediated by IgE antibodies. The ability to induce antigen-specific tolerance provides a powerful avenue to alleviate allergies by exposure to the antigen in conjunction with down-regulation of B7-1, B7-2, B7-3 or CD40.

The specific roles of B7-1, B7-2 and B7-3 in T cell activation remains to be determined. Some studies suggest that their functions are essentially redundant (Hathcock et al 1994 *J Exp. Med.* 180, 631), or that the differences observed in the kinetics of expression might simply indicate that B7-2 is important in the initiation of the co-stimulatory signal, while B7-1 plays a role in the amplification of that signal. Other studies point to more specific functions. For example, Kuchroo et al., 1995 *Cell* 80, 707, have reported that blocking B7-1 expression may favor a Th2 response, while blocking B7-2 expression favors a Th1 response. These two helper T cell subpopulations play distinct roles in the immune response and inflammatory disease. Th1 cells are

strongly correlated with auto-immune disease. Allergic responses are typically triggered by Th2 response. Therefore, the decision to target B7-1, B7-2, CD40 or a combination of the above will depend to the particular disease application.

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Summary of the Invention

Applicant notes that the inhibition of collagenase and stromelysin production in the synovial membrane of joints can be accomplished using ribozymes and antisense molecules. Ribozyme treatment can be a partner to
10 current treatments which primarily target immune cells reacting to pre-existing tissue damage. Early ribozyme or antisense treatment which reduces the collagenase or stromelysin-induced damage can be followed by treatment with the anti-inflammatories or retinoids, if necessary. In this manner, expression of the proteinases can be controlled at both transcriptional and
15 translational levels. Ribozyme or antisense treatment can be given to patients expressing radiological signs of osteoarthritis prior to the expression of clinical symptoms. Ribozyme or antisense treatment can impact the expression of stromelysin without introducing the non-specific effects upon gene expression which accompany treatment with the retinoids and dexamethasone. The
20 ability of stromelysin to activate procollagenase indicates that a ribozyme or antisense molecule which reduces stromelysin expression can also be used in the treatment of both osteoarthritis (which is primarily a stromelysin-associated pathology) and rheumatoid arthritis (which is primarily related to enhanced collagenase activity).

25 While a number of cytokines and growth factors induce metalloproteinase activities during wound healing and tissue injury of a pre-osteoarthritic condition, these molecules are not preferred targets for therapeutic intervention. Primary emphasis is placed upon inhibiting the molecules which are responsible for the disruption of the extracellular matrix, because most people will be presenting radiologic or clinical symptoms prior
30 to treatment. The most versatile of the metalloproteinases (the molecule which can do the most structural damage to the extracellular matrix, if not regulated)

is stromelysin. Additionally, this molecule can activate procollagenase, which in turn causes further damage to the collagen backbone of the extracellular matrix. Under normal conditions, the conversion of prostromelysin to active stromelysin is regulated by the presence of inhibitors called TIMPs (tissue inhibitors of MMP). Because the level of TIMP in synovial cells exceeds the level of prostromelysin and stromelysin activity is generally absent from the synovial fluid associated with non-arthritis tissues, the toxic effects of inhibiting stromelysin activity in non-target cells should be negligible.

Thus, the invention features use of specific ribozyme molecules to treat or prevent arthritis, particularly osteoarthritis, by inhibiting the synthesis of the prostromelysin molecule in synovial cells, or by inhibition of other matrix metalloproteinases discussed above. Cleavage of targeted mRNAs (stromelysin mRNAs, including stromelysin 1, 2, and 3, and collagenase) expressed in macrophages, neutrophils and synovial cells represses the synthesis of the zymogen form of stromelysin, prostromelysin.

Ribozymes are RNA molecules having an enzymatic activity which is able to repeatedly cleave other separate RNA molecules in a nucleotide base sequence specific manner. It is said that such enzymatic RNA molecules can be targeted to virtually any RNA transcript and efficient cleavage has been achieved *in vitro*. Kim et al., 84 Proc. Nat. Acad. of Sci. USA 8788, 1987; Haseloff and Gerlach, 334 Nature 585, 1988; Cech, 260 JAMA 3030, 1988; and Jefferies et al., 17 Nucleic Acid Research 1371, 1989.

Six basic varieties of naturally-occurring enzymatic RNAs are known presently. Each can catalyze the hydrolysis of RNA phosphodiester bonds in *trans* (and thus can cleave other RNA molecules) under physiological conditions. Table I summarizes some of the characteristics of these ribozymes. In general, enzymatic nucleic acids act by first binding to a target RNA. Such binding occurs through the target binding portion of a enzymatic nucleic acid which is held in close proximity to an enzymatic portion of the molecule that acts to cleave the target RNA. Thus, the enzymatic nucleic acid first recognizes and then binds a target RNA through complementary base-pairing, and once bound to the correct site, acts enzymatically to cut the target RNA. Strategic cleavage of such a target RNA will destroy its ability to direct

synthesis of an encoded protein. After an enzymatic nucleic acid has bound and cleaved its RNA target, it is released from that RNA to search for another target and can repeatedly bind and cleave new targets.

By "enzymatic RNA molecule" it is meant an RNA molecule which has
5 complementarity in a substrate binding region to a specified mRNA target, and also has an enzymatic activity which is active to specifically cleave that mRNA. That is, the enzymatic RNA molecule is able to intermolecularly cleave mRNA and thereby inactivate a target mRNA molecule. This complementarity functions to allow sufficient hybridization of the enzymatic RNA molecule to the
10 target RNA to allow the cleavage to occur. One hundred percent complementarity is preferred, but complementarity as low as 50-75% may also be useful in this invention. For *in vivo* treatment, complementarity between 30 and 45 bases is preferred; although lower numbers are also useful.

By "complementary" is meant a nucleotide sequence that can form
15 hydrogen bond(s) with other nucleotide sequence by either traditional Watson-Crick or other non-traditional types (for example Hoogsteen type) of base-paired interactions.

The enzymatic nature of a ribozyme is advantageous over other technologies, such as antisense technology (where a nucleic acid molecule
20 simply binds to a nucleic acid target to block its translation) since the concentration of ribozyme necessary to affect a therapeutic treatment is lower than that of an antisense oligonucleotide. This advantage reflects the ability of the ribozyme to act enzymatically. Thus, a single ribozyme molecule is able to cleave many molecules of target RNA. In addition, the ribozyme is a highly
25 specific inhibitor, with the specificity of inhibition depending not only on the base pairing mechanism of binding to the target RNA, but also on the mechanism of target RNA cleavage. Single mismatches, or base-substitutions, near the site of cleavage can completely eliminate catalytic activity of a ribozyme. Similar mismatches in antisense molecules do not
30 prevent their action (Woolf, T. M., et al., 1992, Proc. Natl. Acad. Sci. USA, 89, 7305-7309). Thus, the specificity of action of a ribozyme is greater than that of an antisense oligonucleotide binding the same RNA site.

In preferred embodiments of this invention, the enzymatic nucleic acid molecule is formed in a hammerhead or hairpin motif, but may also be formed in the motif of a hepatitis delta virus, group I intron or RNaseP RNA (in association with an RNA guide sequence) or *Neurospora* VS RNA. Examples of such hammerhead motifs are described by Rossi *et al.*, 1992, Aids Research and Human Retroviruses 8, 183, of hairpin motifs by Hampel *et al.*, EPA 0360257, Hampel and Tritz, 1989 Biochemistry 28, 4929, and Hampel *et al.*, 1990 Nucleic Acids Res. 18, 299, and an example of the hepatitis delta virus motif is described by Perrotta and Been, 1992 Biochemistry 31, 16; of the RNaseP motif by Guerrier-Takada *et al.*, 1983 Cell 35, 849, *Neurospora* VS RNA ribozyme motif is described by Collins (Saville and Collins, 1990 Cell 61, 685-696; Saville and Collins, 1991 Proc. Natl. Acad. Sci. USA 88, 8826-8830; Collins and Olive, 1993 Biochemistry 32, 2795-2799) and of the Group I intron by Cech *et al.*, U.S. Patent 4,987,071. These specific motifs are not limiting in the invention and those skilled in the art will recognize that all that is important in an enzymatic nucleic acid molecule of this invention which is complementary to one or more of the target gene RNA regions, and that it have nucleotide sequences within or surrounding that substrate binding site which impart an RNA cleaving activity to the molecule.

The invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target stromelysin encoding mRNAs such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA or RNA vectors that are delivered to specific cells.

Synthesis of nucleic acids greater than 100 nucleotides in length is difficult using automated methods, and the therapeutic cost of such molecules is prohibitive. In this invention, small enzymatic nucleic acid motifs (*e.g.*, of the hammerhead or the hairpin structure) are used for exogenous delivery. The simple structure of these molecules increases the ability of the enzymatic nucleic acid to invade targeted regions of the mRNA structure. However,

these catalytic RNA molecules can also be expressed within cells from eukaryotic promoters (e.g., Scanlon et al., 1991, Proc. Natl. Acad. Sci. USA, 88, 10591-5; Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Dropulic et al., 1992 J. Virol., 66, 1432-41; Weerasinghe et al., 1991 J. Virol., 65, 5531-4; Ojwang et al., 1992 Proc. Natl. Acad. Sci. USA, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Sarver et al., 1990 Science 247, 1222-1225; Thompson et al., 1995 Nucleic Acids Res., 23, 2259). Those skilled in the art realize that any ribozyme can be expressed in eukaryotic cells from the appropriate DNA vector. The activity of such ribozymes can be augmented by their release from the primary transcript by a second ribozyme (Draper et al., PCT WO 93/23569, and Sullivan et al., PCT WO 94/02595; Ohkawa et al., 1992 Nucleic Acids Symp. Ser., 27, 15-6; Taira et al., 1991, Nucleic Acids Res., 19, 5125-30; Ventura et al., 1993 Nucleic Acids Res., 21, 3249-55; Chowrira et al., 1994 J. Biol. Chem., 269, 25856) .

15 Ribozyme therapy, due to its exquisite specificity, is particularly well-suited to target mRNA encoding factors that contribute to disease pathology. Thus, ribozymes that cleave stromelysin mRNAs may represent novel therapeutics for the treatment of asthma.

20 Thus, in a first aspect, the invention features ribozymes that inhibit stromelysin production. These chemically or enzymatically synthesized RNA molecules contain substrate binding domains that bind to accessible regions of their target mRNAs. The RNA molecules also contain domains that catalyze the cleavage of RNA. The RNA molecules are preferably ribozymes of the hammerhead or hairpin motif. Upon binding, the ribozymes cleave the target
25 stromelysin encoding mRNAs, preventing translation and stromelysin protein accumulation. In the absence of the expression of the target gene, a therapeutic effect may be observed.

30 By "inhibit" is meant that the activity or level of stromelysin encoding mRNAs and protein is reduced below that observed in the absence of the ribozyme, and preferably is below that level observed in the presence of an inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the level of stromelysin activity in a cell or tissue. By "related" is meant that the inhibition of stromelysin mRNAs and thus reduction in the level of stromelysin activity will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The RNA or RNA complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, aerosol inhalation, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables AII, AIII, AIV, AVI, AVIII and AIX. Examples of such ribozymes are shown in Tables AV, AVII, AVIII and AIX. Examples of such ribozymes consist essentially of sequences defined in these Tables.

By "consists essentially of" is meant that the active ribozyme contains an enzymatic center equivalent to those in the examples, and binding arms able to bind mRNA such that cleavage at the target site occurs. Other sequences may be present which do not interfere with such cleavage.

In a related aspect the invention features ribozymes that cleave target molecules and inhibit stromelysin activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for introduction into the desired target cell.

By "vectors" is meant any nucleic acid- and/or viral-based technique used to deliver a desired nucleic acid.

This class of chemicals exhibits a high degree of specificity for cleavage of the intended target mRNA. Consequently, the ribozyme agent will only
5 affect cells expressing that particular gene, and will not be toxic to normal tissues.

The invention can be used to treat or prevent (prophylactically) osteoarthritis or other pathological conditions which are mediated by metalloproteinase activation. The preferred administration protocol is *in vivo*
10 administration to reduce the level of stromelysin activity.

Thus, the invention features an enzymatic RNA molecule (or ribozyme) which cleaves mRNA associated with development or maintenance of an arthritic condition, e.g., mRNA encoding stromelysin, and in particular, those
15 mRNA targets disclosed in the accompanying tables, which include both hammerhead and hairpin target sites. In each case the site is flanked by regions to which appropriate substrate binding arms can be synthesized and an appropriate hammerhead or hairpin motif can be added to provide enzymatic activity, by methods described herein and known in the art. For example, referring to Figure 1, arms I and III are modified to be specific
20 substrate-binding arms, and arm II remains essentially as shown.

Ribozymes that cleave stromelysin mRNAs represent a novel therapeutic approach to arthritic disorders like osteoarthritis. The invention features use of ribozymes to treat osteoarthritis, e.g., by inhibiting the synthesis of prostromelysin molecule in synovial cells or by the inhibition of matrix
25 metalloproteinases. Applicant indicates that ribozymes are able to inhibit the secretion of stromelysin and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave stromelysin encoding mRNAs may be readily designed and are within the
30 invention.

In other related aspects, the invention features a mammalian cell which includes an enzymatic RNA molecule as described above. Preferably, the

mammalian cell is a human cell; and the invention features an expression vector which includes nucleic acid encoding an enzymatic RNA molecule described above, located in the vector, e.g., in a manner which allows expression of that enzymatic RNA molecule within a mammalian cell; or a
5 method for treatment of a disease or condition by administering to a patient an enzymatic RNA molecule as described above.

The invention provides a class of chemical cleaving agents which exhibit a high degree of specificity for the mRNA causative of an arthritic condition. Such enzymatic RNA molecules can be delivered exogenously or
10 endogenously to infected cells. In the preferred hammerhead motif the small size (less than 40 nucleotides, preferably between 32 and 36 nucleotides in length) of the molecule allows the cost of treatment to be reduced.

The enzymatic RNA molecules of this invention can be used to treat arthritic or prearthritic conditions. Such treatment can also be extended to
15 other related genes in nonhuman primates. Affected animals can be treated at the time of arthritic risk detection, or in a prophylactic manner. This timing of treatment will reduce the chance of further arthritic damage.

In another aspect, the invention features novel nucleic acid-based techniques [e.g., enzymatic nucleic acid molecules (ribozymes), antisense
20 nucleic acids, 2-5A antisense chimeras, triplex DNA, antisense nucleic acids containing RNA cleaving chemical groups (Cook et al., U.S. Patent 5,359,051)] and methods for their use to induce graft tolerance, to treat autoimmune diseases such as lupus, rheumatoid arthritis, multiple sclerosis and to treatment of allergies.

25 In a preferred embodiment, the invention features use of one or more of the nucleic acid-based techniques to induce graft tolerance by inhibiting the synthesis of B7-1, B7-2, B7-3 and CD40 proteins.

Those in the art will recognize the other potential targets, for e.g., ICAM-1, VCAM-1, β 1 integrin (VLA4) are also suitable for treatment with the nucleic
30 acid-based techniques described in the present invention.

By "inhibit" is meant that the activity of B7-1, B7-2, B7-3 and/or CD40 or level of mRNAs encoded by B7-1, B7-2, B7-3 and/or CD40 is reduced below that observed in the absence of the nucleic acid. In one embodiment, inhibition with ribozymes preferably is below that level observed in the presence of an enzymatically inactive RNA molecule able to bind to the same site on the mRNA, but unable to cleave that RNA.

By "equivalent" RNA to B7-1, B7-2, B7-3 and/or CD40 is meant to include those naturally occurring RNA molecules associated with graft rejection in various animals, including human, mice, rats, rabbits, primates and pigs.

By "antisense nucleic acid" is meant a non-enzymatic nucleic acid molecule that binds to another RNA (target RNA) by means of RNA-RNA or RNA-DNA or RNA-PNA (protein nucleic acid; Egholm et al., 1993 *Nature* 365, 566) interactions and alters the activity of the target RNA (for a review see Stein and Cheng, 1993 *Science* 261, 1004).

By "2-5A antisense chimera" is meant, an antisense oligonucleotide containing a 5' phosphorylated 2'-5'-linked adenylyate residues. These chimeras bind to target RNA in a sequence-specific manner and activate a cellular 2-5A-dependent ribonuclease which in turn cleaves the target RNA (Torrence et al., 1993 *Proc. Natl. Acad. Sci. USA* 90, 1300).

By "triplex DNA" is meant an oligonucleotide that can bind to a double-stranded DNA in a sequence-specific manner to form a triple-strand helix. Triple-helix formation has been shown to inhibit transcription of the targeted gene (Duval-Valentin et al., 1992 *Proc. Natl. Acad. Sci. USA* 89, 504).

By "gene" is meant a nucleic acid that encodes an RNA.

Ribozymes that cleave the specified sites in B7-1, B7-2, B7-3 and/or CD40 mRNAs represent a novel therapeutic approach to induce graft tolerance and treat autoimmune diseases, allergies and other inflammatory conditions. Applicant indicates that ribozymes are able to inhibit the activity of B7-1, B7-2, B7-3 and/or CD40 and that the catalytic activity of the ribozymes is required for their inhibitory effect. Those of ordinary skill in the art, will find that it is clear from the examples described that other ribozymes that cleave these

sites in B7-1, B7-2, B7-3 and/or CD40 mRNAs may be readily designed and are within the invention.

In a preferred embodiment the invention provides a method for producing a class of enzymatic cleaving agents which exhibit a high degree of specificity for the RNA of a desired target. The enzymatic nucleic acid molecule is preferably targeted to a highly conserved sequence region of a target mRNAs encoding B7-1, B7-2, B7-3 and/or CD40 proteins such that specific treatment of a disease or condition can be provided with either one or several enzymatic nucleic acids. Such enzymatic nucleic acid molecules can be delivered exogenously to specific cells as required. Alternatively, the ribozymes can be expressed from DNA/RNA vectors that are delivered to specific cells.

Such ribozymes are useful for the prevention of the diseases and conditions discussed above, and any other diseases or conditions that are related to the levels of B7-1, B7-2, B7-3 and/or CD40 activity in a cell or tissue. By "related" is meant that the inhibition of B7-1, B7-2, B7-3 and/or CD40 mRNAs and thus reduction in the level respective protein activity will relieve to some extent the symptoms of the disease or condition.

Ribozymes are added directly, or can be complexed with cationic lipids, packaged within liposomes, or otherwise delivered to target cells. The nucleic acid or nucleic acid complexes can be locally administered to relevant tissues ex vivo, or in vivo through injection, infusion pump or stent, with or without their incorporation in biopolymers. In preferred embodiments, the ribozymes have binding arms which are complementary to the sequences in Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Examples of such ribozymes are shown in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Examples of such ribozymes consist essentially of sequences defined in these Tables.

In another aspect of the invention, ribozymes that cleave target molecules and inhibit B7-1, B7-2, B7-3 and/or CD40 activity are expressed from transcription units inserted into DNA or RNA vectors. The recombinant vectors are preferably DNA plasmids or viral vectors. Ribozyme expressing viral vectors could be constructed based on, but not limited to, adeno-

associated virus, retrovirus, adenovirus, or alphavirus. Preferably, the recombinant vectors capable of expressing the ribozymes are delivered as described above, and persist in target cells. Alternatively, viral vectors may be used that provide for transient expression of ribozymes. Such vectors might be
5 repeatedly administered as necessary. Once expressed, the ribozymes cleave the target mRNA. Delivery of ribozyme expressing vectors could be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that would allow for
10 introduction into the desired target cell.

Other features and advantages of the invention will be apparent from the following description of the preferred embodiments thereof, and from the claims.

15 Description of the Preferred Embodiments

The drawings will first briefly be described.

Drawings

Figure 1 is a diagrammatic representation of the hammerhead ribozyme domain known in the art. Stem II can be ≥ 2 base-pairs long.

20 Figure 2a is a diagrammatic representation of the hammerhead ribozyme domain known in the art; Figure 2b is a diagrammatic representation of the hammerhead ribozyme as divided by Uhlenbeck (1987, *Nature*, 327, 596-600) into a substrate and enzyme portion; Figure 2c is a similar diagram showing the hammerhead divided by Haseloff and Gerlach (1988, *Nature*, 334, 585-
25 591) into two portions; and Figure 2d is a similar diagram showing the hammerhead divided by Jeffries and Symons (1989, *Nucl. Acids. Res.*, 17, 1371-1371) into two portions.

Figure 3 is a diagrammatic representation of the general structure of a hairpin ribozyme. Helix 2 (H2) is provided with a least 4 base pairs (*i.e.*, *n* is
30 1, 2, 3 or 4) and helix 5 can be optionally provided of length 2 or more bases

(preferably 3 - 20 bases, *i.e.*, *m* is from 1 - 20 or more). Helix 2 and helix 5 may be covalently linked by one or more bases (*i.e.*, *r* is ≥ 1 base). Helix 1, 4 or 5 may also be extended by 2 or more base pairs (*e.g.*, 4 - 20 base pairs) to stabilize the ribozyme structure, and preferably is a protein binding site. In each instance, each N and N' independently is any normal or modified base and each dash represents a potential base-pairing interaction. These nucleotides may be modified at the sugar, base or phosphate. Complete base-pairing is not required in the helices, but is preferred. Helix 1 and 4 can be of any size (*i.e.*, *o* and *p* is each independently from 0 to any number, *e.g.*, 20) as long as some base-pairing is maintained. Essential bases are shown as specific bases in the structure, but those in the art will recognize that one or more may be modified chemically (abasic, base, sugar and/or phosphate modifications) or replaced with another base without significant effect. Helix 4 can be formed from two separate molecules, *i.e.*, without a connecting loop. The connecting loop when present may be a ribonucleotide with or without modifications to its base, sugar or phosphate. "q" is ≥ 2 bases. The connecting loop can also be replaced with a non-nucleotide linker molecule. H, refers to bases A, U or C. Y refers to pyrimidine bases. " - " refers to a chemical bond.

Figure 4 is a representation of the general structure of the hepatitis delta virus ribozyme domain known in the art.

Figure 5 is a representation of the general structure of the self-cleaving VS RNA ribozyme domain.

Figure 6 is a schematic representation of an RNaseH accessibility assay. Specifically, the left side of Figure 6 is a diagram of complementary DNA oligonucleotides bound to accessible sites on the target RNA. Complementary DNA oligonucleotides are represented by broad lines labeled A, B, and C. Target RNA is represented by the thin, twisted line. The right side of Figure 6 is a schematic of a gel separation of uncut target RNA from a cleaved target RNA. Detection of target RNA is by autoradiography of body-labeled, T7 transcript. The bands common to each lane represent uncleaved target RNA; the bands unique to each lane represent the cleaved products.

Figure 7 shows *in vitro* cleavage of stromelysin mRNA by HH ribozymes.

Figure 8 shows inhibition of stromelysin expression by 21HH ribozyme in HS-27 fibroblast cell line.

Figure 9 shows inhibition of stromelysin expression by 463HH ribozyme
5 in HS-27 fibroblast cell line.

Figure 10 shows inhibition of stromelysin expression by 1049HH ribozyme in HS-27 fibroblast cell line.

Figure 11 shows inhibition of stromelysin expression by 1366HH ribozyme in HS-27 fibroblast cell line.

10 Figure 12 shows inhibition of stromelysin expression by 1410HH ribozyme in HS-27 fibroblast cell line.

Figure 13 shows inhibition of stromelysin expression by 1489HH ribozyme in HS-27 fibroblast cell line.

15 Figure 14 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 15 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 16 shows 1049HH ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

20 Figure 17 shows the effect of phosphorothioate substitutions on the catalytic activity of 1049 2'-C-allyl HH ribozyme. A) diagrammatic representation of 1049 hammerhead ribozyme•substrate complex. 1049 U4-C-allyl P=S ribozyme represents a hammerhead containing ribose residues at five positions. The remaining 31 nucleotide positions contain 2'-hydroxyl
25 group substitutions, wherein 30 nucleotides contain 2'-O-methyl substitutions and one nucleotide (U₄) contains 2'-C-allyl substitution. Additionally, five nucleotides within the ribozyme, at the 5' and 3' termini, contain

phosphorothioate substitutions. B) shows the ability of ribozyme described in Fig. 17A to decrease the level of stromelysin RNA in rabbit knee.

Figure 18 is a diagrammatic representation of chemically modified ribozymes targeted against stromelysin RNA. 1049 2'-amino P=S Ribozyme
5 represents a hammerhead containing ribose residues at five positions. The remaining 31 nucleotide positions contain 2'-hydroxyl group substitutions, wherein 29 nucleotides contain 2'-O-methyl substitutions and two nucleotides (U₄ and U₇) contain 2'-amino substitution. Additionally, the 3' end of this ribozyme contains a 3'-3' linked inverted T and four nucleotides at the 5'
10 termini contain phosphorothioate substitutions. Arrow-head indicates the site of RNA cleavage (site 1049). 1363 2'-Amino P=S, Human and Rabbit 1366 2'-Amino P=S ribozymes are identical to the 1049 2'-amino P=S ribozyme except that they are targeted to sites 1363 and 1366 within stromelysin RNAs.

Figure 19 shows 1049 2'-amino P=S ribozyme-mediated reduction in the
15 level of stromelysin mRNA in rabbit knee.

Figure 20 shows 1363 2'-amino P=S ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

Figure 21 shows 1366 2'-amino P=S ribozyme-mediated reduction in the level of stromelysin mRNA in rabbit knee.

20 Figures 22a-d are diagrammatic representations of non-limiting examples of base modifications for adenine, guanine, cytosine and uracil, respectively.

Figure 23 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al.*, *Nucleic Acids Res.* 1992,
25 20:3252) showing specific substitutions in the catalytic core and substrate binding arms. Compounds 4, 9, 13, 17, 22 and 23 are described in Fig. 24.

Figure 24 is a diagrammatic representation of various nucleotides that can be substituted in the catalytic core of a hammerhead ribozyme.

Figure 25 is a diagrammatic representation of the synthesis of a
30 ribothymidine phosphoramidite.

Figure 26 is a diagrammatic representation of the synthesis of a 5-methylcytidine phosphoramidite.

Figure 27 is a diagrammatic representation of the synthesis of 5-bromouridine phosphoramidite.

5 Figure 28 is a diagrammatic representation of the synthesis of 6-azauridine phosphoramidite.

Figure 29 is a diagrammatic representation of the synthesis of 2,6-diaminopurine phosphoramidite.

10 Figure 30 is a diagrammatic representation of the synthesis of a 6-methyluridine phosphoramidite.

Figure 31 is a representation of a hammerhead ribozyme targeted to site A (HH-A). Site of 6-methyl U substitution is indicated.

15 Figure 32 shows RNA cleavage reaction catalyzed by HH-A ribozyme containing 6-methyl U-substitution (6-methyl-U4). U4, represents a HH-A ribozyme containing no 6-methyl-U substitution.

Figure 33 is a representation of a hammerhead ribozyme targeted to site B (HH-B). Sites of 6-methyl U substitution are indicated.

20 Figure 34 shows RNA cleavage reaction catalyzed by HH-B ribozyme containing 6-methyl U-substitutions at U4 and U7 positions (6-methyl-U4). U4, represents a HH-B ribozyme containing no 6-methyl-U substitution.

Figure 35 is a representation of a hammerhead ribozyme targeted to site C (HH-C). Sites of 6-methyl U substitution are indicated.

25 Figure 36 shows RNA cleavage reaction catalyzed by HH-C ribozyme containing 6-methyl U-substitutions at U4 and U7 positions (6-methyl-U4). U4, represents a HH-C ribozyme containing no 6-methyl-U substitution.

Figure 37 shows 6-methyl-U-substituted HH-A ribozyme-mediated inhibition of rat smooth muscle cell proliferation.

Figure 38 shows 6-methyl-U-substituted HH-C ribozyme-mediated inhibition of stromelysin protein production in human synovial fibroblast cells.

Figure 39 is a diagrammatic representation of the synthesis of pyridin-2-one nucleoside and pyridin-4-one nucleoside phosphoramidite.

- 5 Figure 40 is a diagrammatic representation of the synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-3-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-phenyl- β -D-ribofuranose phosphoramidite.

- 10 Figure 41 is a diagrammatic representation of the synthesis of pseudouridine, 2,4,6-trimethoxy benzene nucleoside and 3-methyluridine phosphoramidite.

Figure 42 is a diagrammatic representation of the synthesis of dihydrouridine phosphoramidite.

- 15 Figure 43 A) is diagrammatic representation of a hammerhead ribozyme targeted to site B. B) shows RNA cleavage reaction catalyzed by hammerhead ribozyme with modified base substitutions at either position 4 or position 7.

- 20 Figure 44 shows further kinetic characterization of RNA cleavage reactions catalyzed by HH-B ribozyme (A); HH-B with pyridin-4-one substitution at position 7 (B); and HH-B with phenyl substitution at position 7 (C).

Figure 45 is a diagrammatic representation of the synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-Deoxy-1-Naphthyl- β -D-Ribofuranose.

- 25 Figure 46 is a diagrammatic representation of the synthesis of Synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-Deoxy-1-(*p*-Aminophenyl)- β -D-Ribofuranose.

Figure 47 is a diagrammatic representation of a position numbered hammerhead ribozyme (according to Hertel *et al. Nucleic Acids Res.* 1992, 20, 3252) showing specific substitutions.

Figure 48 shows the structures of various 2'-alkyl modified nucleotides which exemplify those of this invention. R groups are alkyl groups, Z is a protecting group.

Figure 49 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine.

Figure 50 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene uridine.

Figure 51 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene cytidine.

Figure 52 is a diagrammatic representation of the synthesis of 2'-C-methylene and 2'-C-difluoromethylene adenosine.

Figure 53 is a diagrammatic representation of the synthesis of 2'-C-carboxymethylidene uridine, 2'-C-methoxycarboxymethylidene uridine and derivatized amidites thereof. X is CH₃ or alkyl as discussed above, or another substituent.

Figure 54 is a diagrammatic representation of the synthesis of 2'-C-allyl uridine and cytidine phosphoramidites.

Figure 55 is a diagrammatic representation of the synthesis of 2'-O-alkylthioalkyl nucleosides or non-nucleosides and their phosphoramidites. R is an alkyl as defined above. B is any naturally occurring or modified base bearing any N-protecting group suitable for standard oligonucleotide synthesis (Usman *et al.*, *supra*; Scaringe *et al.*, *supra*), and/or H (non-nucleotide), as described by the publications discussed above. CE is cyanoethyl, DMT is a standard blocking group. Other abbreviations are standard in the art.

Figure 56 is a diagrammatic representation of a hammerhead ribozyme, targeted to site B (HH-B), containing 2'-O-methylthiomethyl substitutions.

Figure 57 shows RNA cleavage activity catalyzed by 2'-O-methylthiomethyl substituted ribozymes. A plot of percent cleaved as a function of time is shown. The reactions were carried out at 37°C in the presence of 40 nM ribozyme, 1 nM substrate and 10 mM MgCl₂. Control HH-B ribozyme contained the following modifications; 29 positions were modified with 2'-O-methyl, U4 and U7 positions were modified with 2'-amino groups, 5 positions contained 2'-OH groups. These modifications of the control ribozyme have previously been shown not to significantly effect the activity of the ribozyme (Usman et al., 1994 *Nucleic Acids Symposium Series* 31, 163).

Figure 58 is a diagrammatic representation of the synthesis of an abasic deoxyribose or ribose non-nucleotide mimetic phosphoramidite.

Figure 59 is a diagrammatic representation of a hammerhead ribozyme targeted to site B (HH-B). Arrow indicates the cleavage site.

Figure 60 is a diagrammatic representation of HH-B ribozyme containing abasic substitutions (HH-Ba) at various positions. Ribozymes were synthesized as described in the application. "X" shows the positions of abasic substitutions. The abasic substitutions were either made individually or in certain combinations.

Figure 61 shows the *in vitro* RNA cleavage activity of HH-B and HH-Ba ribozymes. All RNA, refers to HHA ribozyme containing no abasic substitution. U4 Abasic, refers to HH-Ba ribozyme with a single abasic (ribose) substitution at position 4. U7 Abasic, refers to HH-Ba ribozyme with a single abasic (ribose) substitution at position 7.

Figure 62 shows *in vitro* RNA cleavage activity of HH-B and HH-Ba ribozymes. Abasic Stem II Loop, refers to HH-Ba ribozyme with four abasic (ribose) substitutions within the loop in stem II.

Figure 63 shows *in vitro* RNA cleavage activity of HH-B and HH-Ba ribozymes. 3'-Inverted Deoxyribose, refers to HH-Ba ribozyme with an inverted deoxyribose (abasic) substitution at its 3' termini.

Figure 64 is a diagrammatic representation of a hammerhead ribozyme
5 targeted to site A (HH-A). Target A is involved in the proliferation of mammalian smooth muscle cells. Arrow indicates the site of cleavage. Inactive version of HH-A contains 2 base-substitutions (G5U and A15.1U) that renders the ribozyme catalytically inactive.

Figure 65 is a diagrammatic representation of HH-A ribozyme with abasic
10 substitution (HH-Aa) at position 4. X, shows the position of abasic substitution.

Figure 66 shows ribozyme-mediated inhibition of rat aortic smooth muscle cell (RASMC) proliferation. Both HH-A and HH-Aa ribozymes can inhibit the proliferation of RASMC in culture. Catalytically inactive HH-A ribozyme shows inhibition which is significantly lower than active HH-A and
15 HH-Aa ribozymes.

Figure 67 is a schematic representation of a two pot deprotection protocol with ethylamine (EA).

Figure 68 shows a strategy used in synthesizing a hammerhead ribozyme from two halves. X and Y represent reactive moieties that can
20 undergo a chemical reaction to form a covalent bond (represented by the solid curved line).

Figure 69 shows various non-limiting examples of reactive moieties that can be placed in the nascent loop region to form a covalent bond to provide a full-length ribozyme. CH₂ can be any linking chain as described above
25 including groups such as methylenes, ether, ethylene glycol, thioethers, double bonds, aromatic groups and others; each n independently is an integer from 0 to 10 inclusive and may be the same or different; each R independently is a proton or an alkyl, alkenyl and other functional groups or conjugates such as peptides, steroids, hormones, lipids, nucleic acid sequences and others
30 that provides nuclease resistance, improved cell association, improved cellular uptake or interacellular localization.

Figure 70 shows non-limiting examples of covalent bonds that can be formed to provide the full length ribozyme. The morpholino group arises from reductive reaction of a dialdehyde, which arises from oxidative cleavage of a ribose at the 3'-end of one half ribozyme with an amine at the 5'-end of the half ribozyme. The amide bond is produced when an acid at the 3'-end of one half ribozyme is coupled to an amine at the 5'-end of the other half ribozyme.

Figure 71 shows non-limiting examples of three ribozymes that were synthesized from coupling reactions of two halves. All three were targeted to the site A of *c-myc* RNA (HH-A). HH-A1 was formed from the reaction of two thiols to provide the disulfide linked ribozyme. HH-A2 and HH-A3 were formed using the morpholino reaction. HH-A2 contains a five atom spacer linking the terminal amine to the 5'-end of the half ribozyme. HH-A3 contains a six carbon spacer linking the terminal amine to the 5'-end of the half ribozyme.

Figure 72 shows comparative cleavage activity of half ribozymes, containing five and six base pair stem II regions, that are not covalently linked vs a full length ribozyme. Assays were carried out under ribozyme excess conditions.

Figure 73 shows comparative cleavage activity of half ribozymes, containing seven and eight base pair stem II regions, that are not covalently linked vs a full length ribozyme. Assays were carried out under ribozyme excess conditions.

Figure 74 shows comparative cleavage assay of HH-A1, HH-A2 and HH-A3 (see Figure 72) formed from crosslinking reactions vs a full length ribozyme control. Assays were carried out under ribozyme excess conditions.

Figure 75. Schematic representation of RNA polymerase III promoter structure. Arrow indicates the transcription start site and the direction of coding region. A, B and C, refer to consensus A, B and C box promoter sequences. I, refers to intermediate cis-acting promoter sequence. PSE, refers to proximal sequence element. DSE, refers to distal sequence element. ATF, refers to activating transcription factor binding element. ?, refers to cis-

acting sequence element that has not been fully characterized. EBER, Epstein-Barr-virus-encoded-RNA. TATA is a box well known in the art.

Figure 76 is a general formula for pol III RNA of this invention.

5 Figure 77 is a diagrammatic representation of a U6-S35 Chimera. The S35 motif and the site of insertion of a desired RNA are indicated. This chimeric RNA transcript is under the control of a U6 small nuclear RNA (snRNA) promoter.

10 Figure 78 is a diagrammatic representation of a U6-S35-ribozyme chimera. The chimera contains a hammerhead ribozyme targeted to site I (HHI).

Figure 79 is a diagrammatic representation of a U6-S35-ribozyme chimera. The chimera contains a hammerhead ribozyme targeted to site II (HHII).

15 Figure 80 shows RNA cleavage reaction catalyzed by a synthetic hammerhead ribozyme (HHI) and by an *in vitro* transcript of U6-S35-HHI hammerhead ribozyme.

Figure 81 shows stability of U6-S35-HHII RNA transcript in 293 mammalian cells as measured by actinomycin D assay.

20 Figure 82 is a diagrammatic representation of an adenovirus VA1 RNA. Various domains within the RNA secondary structure are indicated.

25 Figure 83 A shows a secondary structure model of a VA1-S35 chimeric RNA containing the promoter elements A and B box. The site of insertion of a desired RNA and the S35 motif are indicated. The transcription unit also contains a stable stem (S35-like motif) in the central domain of the VA1 RNA which positions the desired RNA away from the main transcript as an independent domain. 83B shows a VA1-chimera which consists of the terminal 75 nt of a VA1 RNA followed by the HHI ribozyme.

Figure 84 shows a comparison of stability of VA1-chimeric RNA vs VA1-S35-chimeric RNA as measured by actinomycin D assay. VA1-chimera

consists of terminal 75 nt of VA1 RNA followed by HHI ribozyme. VA1-S35-chimera structure and sequence is shown in Figure 83.

Ribozymes

5 Ribozymes in one aspect of this invention block to some extent stromelysin expression and can be used to treat disease or diagnose such disease. Ribozymes are delivered to cells in culture and to cells or tissues in animal models of osteoarthritis (Hembry et al., 1993 Am. J. Pathol. 143, 628). Ribozyme cleavage of stromelysin encoding mRNAs in these systems may prevent inflammatory cell function and alleviate disease symptoms.

10 Other ribozymes of this invention block to some extent B7-1, B7-2, B7-3 and/or CD40 production and can be used to treat disease or diagnose such disease. Ribozymes will be delivered to cells in culture, to cells or tissues in animal models of transplantation, autoimmune diseases and/or allergies and to human cells or tissues *ex vivo* or *in vivo*. Ribozyme cleavage of B7-1, B7-2
15 and/or CD40 encoded mRNAs in these systems may alleviate disease symptoms.

Target sites

Targets for useful ribozymes can be determined as disclosed in Draper et al supra, Sullivan et al., supra, as well as by Draper et al., WO 95/13380 and
20 Stinchcomb et al WO 95/23225. Rather than repeat the guidance provided in those documents here, below are provided specific examples of such methods, not limiting to those in the art. Ribozymes to such targets are designed as described in those applications and synthesized to be tested *in vitro* and *in vivo*, as also described. Such ribozymes can also be optimized
25 and delivered as described therein. While specific examples to mouse, rabbit and other animal RNA are provided, those in the art will recognize that the equivalent human RNA targets described can be used as described below. Thus, the same target may be used, but binding arms suitable for targeting human RNA sequences are present in the ribozyme. Such targets may also
30 be selected as described below.

5 The sequence of human and rabbit stromelysin mRNA were screened for accessible sites using a computer folding algorithm. Potential hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables All, AIII, AIV, AVI, AVIII and AIX (All sequences are 5' to 3' in the tables.). While rabbit and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, rabbit targeted ribozymes are useful to test efficacy of action of the ribozyme prior to testing in humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

10 Similarly, the sequence of human and mouse B7-1, B7-2, B7-3 and/or CD40 mRNAs were screened for optimal ribozyme target sites using a computer folding algorithm. Hammerhead or hairpin ribozyme cleavage sites were identified. These sites are shown in Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX (All sequences are 5' to 3' in the tables) The nucleotide base position is noted in the Tables as that site to be
15 cleaved by the designated type of ribozyme. While mouse and human sequences can be screened and ribozymes thereafter designed, the human targeted sequences are of most utility. However, mouse targeted ribozymes may be useful to test efficacy of action of the ribozyme prior to testing in
20 humans. The nucleotide base position is noted in the Tables as that site to be cleaved by the designated type of ribozyme.

Hammerhead or hairpin ribozymes are designed that could bind and are individually analyzed by computer folding (Jaeger et al., 1989 Proc. Natl. Acad. Sci. USA, 86, 7706-7710) to assess whether the ribozyme sequences
25 fold into the appropriate secondary structure. Those ribozymes with unfavorable intramolecular interactions between the binding arms and the catalytic core are eliminated from consideration. Varying binding arm lengths can be chosen to optimize activity. Generally, at least 5 bases on each arm are able to bind to, or otherwise interact with, the target RNA.

30 Referring to Figure 6, mRNA is screened for accessible cleavage sites by the method described generally in Draper WO 93/23569. Briefly, DNA oligonucleotides representing potential hammerhead or hairpin ribozyme cleavage sites are synthesized. A polymerase chain reaction is used to

generate a substrate for T7 RNA polymerase transcription from human or rabbit stromelysin cDNA clones. Labeled RNA transcripts are synthesized *in vitro* from the two templates. The oligonucleotides and the labeled transcripts are annealed, RNaseH is added and the mixtures are incubated for the designated times at 37°C. Reactions are stopped and RNA separated on sequencing polyacrylamide gels. The percentage of the substrate cleaved is determined by autoradiographic quantitation using a PhosphorImaging system. From these data, hammerhead ribozyme sites are chosen as the most accessible.

10 Ribozymes of the hammerhead or hairpin motif are designed to anneal to various sites in the mRNA message. The binding arms are complementary to the target site sequences described above. The ribozymes are chemically synthesized. The method of synthesis used follows the procedure for normal RNA synthesis as described in Usman et al., 1987 J. Am. Chem. Soc., 109, 7845-7854 and in Scaringe et al., 1990 Nucleic Acids Res., 18, 5433-5441; Wincott et al., 1995 Nucleic Acids Res., 23, 2677, and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. The average stepwise coupling yields were >98%. Inactive ribozymes were synthesized by substituting a U for G5 and a U for A14 (numbering from Hertel et al., 1992 Nucleic Acids Res., 20, 3252). Hairpin ribozymes are synthesized in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 Nucleic Acids Res., 20, 2835-2840). All ribozymes are modified extensively to enhance stability by modification with nuclease resistant groups, for example, 2'-amino, 2'-C-allyl, 2'-fluoro, 2'-O-methyl, 2'-H (for a review see Usman and Cedergren, 1992 TIBS 17, 34 and Beigelman et al., 1995 J. Biol. Chem. 270, 25702). Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Stinchcomb et al, supra) and are resuspended in water.

30 The sequences of the chemically synthesized ribozymes useful in this study are shown in Tables AV, AVII, AVIII and AIX and in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX. Those in the art will recognize that these sequences are representative only of many more such sequences where the enzymatic portion of the ribozyme (all but the

binding arms) is altered to affect activity. For example, stem loop II sequence of hammerhead ribozymes listed in Tables AV and AVII (5'-GGCCGAAAGGCC-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. Similarly, stem-loop AIV sequence of hairpin ribozymes listed in Tables AVI and AVII (5'-CACGUUGUG-3') can be altered (substitution, deletion and/or insertion) to contain any sequence provided, a minimum of two base-paired stem structure can form. The sequences listed in Tables AV, AVII, AVIII and AIX may be formed of ribonucleotides or other nucleotides or non-nucleotides. Such ribozymes are equivalent to the ribozymes described specifically in the Tables.

Optimizing Ribozyme Activity

Ribozyme activity can be optimized as described by Stinchcomb *et al.*, supra. The details will not be repeated here, but include altering the length of the ribozyme binding arms (stems I and III, see Figure 2c), or chemically synthesizing ribozymes with modifications that prevent their degradation by serum ribonucleases (see *e.g.*, Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken *et al.*, 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Stinchcomb *et al.*, supra, Sproat, European Patent Application 92110298.4 and U.S. Patent 5,334,711; Jennings *et al.*, WO 94/13688 and Beigelman *et al.*, supra which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules). Modifications which enhance their efficacy in cells, and removal of stem II bases to shorten RNA synthesis times and reduce chemical requirements.

Sullivan, *et al.*, supra, describes the general methods for delivery of enzymatic RNA molecules. Ribozymes may be administered to cells by a variety of methods known to those familiar to the art, including, but not restricted to, encapsulation in liposomes, by iontophoresis, or by incorporation into other vehicles, such as hydrogels, cyclodextrins, biodegradable nanocapsules, and bioadhesive microspheres. For some

indications, ribozymes may be directly delivered *ex vivo* to cells or tissues with or without the aforementioned vehicles. Alternatively, the RNA/vehicle combination is locally delivered by direct inhalation, by direct injection or by use of a catheter, infusion pump or stent. Other routes of delivery include, but are not limited to, intravascular, intramuscular, subcutaneous or joint injection, aerosol inhalation, oral (tablet or pill form), topical, systemic, ocular, intraperitoneal and/or intrathecal delivery. More detailed descriptions of ribozyme delivery and administration are provided in Sullivan et al., supra and Draper et al., supra which have been incorporated by reference herein.

10 In another preferred embodiment, the ribozyme is administered to the site of B7-1, B7-2, B7-3 and/or CD40 expression (APC) in an appropriate liposomal vesicle. APCs isolated from donor (for example) are treated with the ribozyme preparation (or other nucleic acid therapeutics) *ex vivo* and the treated cells are infused into recipient. Alternatively, cells, tissues or organs are directly treated with nucleic acids of the present invention prior to transplantation into a recipient.

Another means of accumulating high concentrations of a ribozyme(s) within cells is to incorporate the ribozyme-encoding sequences into a DNA expression vector. Transcription of the ribozyme sequences are driven from a promoter for eukaryotic RNA polymerase I (pol I), RNA polymerase II (pol II), or RNA polymerase III (pol III). Transcripts from pol II or pol III promoters will be expressed at high levels in all cells; the levels of a given pol II promoter in a given cell type will depend on the nature of the gene regulatory sequences (enhancers, silencers, etc.) present nearby. Prokaryotic RNA polymerase promoters are also used, providing that the prokaryotic RNA polymerase enzyme is expressed in the appropriate cells (Elroy-Stein and Moss, 1990 Proc. Natl. Acad. Sci. U S A, 87, 6743-7; Gao and Huang 1993 Nucleic Acids Res., 21, 2867-72; Lieber et al., 1993 Methods Enzymol., 217, 47-66; Zhou et al., 1990 Mol. Cell. Biol., 10, 4529-37). Several investigators have demonstrated that ribozymes expressed from such promoters can function in mammalian cells (e.g. Kashani-Sabet et al., 1992 Antisense Res. Dev., 2, 3-15; Ojwang et al., 1992 Proc. Natl. Acad. Sci. U S A, 89, 10802-6; Chen et al., 1992 Nucleic Acids Res., 20, 4581-9; Yu et al., 1993 Proc. Natl. Acad. Sci. U S A, 90, 6340-4; L'Huillier et al., 1992 EMBO J., 11, 4411-8; Lisiewicz et

al., 1993 Proc. Natl. Acad. Sci. U. S. A., 90, 8000-4; Thompson *et al.*, *supra*). The above ribozyme transcription units can be incorporated into a variety of vectors for introduction into mammalian cells, including but not restricted to, plasmid DNA vectors, viral DNA vectors (such as adenovirus or adeno-associated vectors), or viral RNA vectors (such as retroviral or alphavirus vectors).

In a preferred embodiment of the invention, a transcription unit expressing a ribozyme that cleaves stromelysin RNA is inserted into a plasmid DNA vector or an adenovirus DNA virus or adeno-associated virus (AAV) vector. Both viral vectors have been used to transfer genes to the lung and both vectors lead to transient gene expression (Zabner *et al.*, 1993 Cell 75, 207; Carter, 1992 Curr. Opi. Biotech. 3, 533). The adenovirus vector is delivered as recombinant adenoviral particles. The DNA may be delivered alone or complexed with vehicles (as described for RNA above). The recombinant adenovirus or AAV particles are locally administered to the site of treatment, *e.g.*, through incubation or inhalation *in vivo* or by direct application to cells or tissues *ex vivo*.

Specifically useful modifications, optimization and synthetic methods will now be described.

20 Base Modifications

The following discussion of relevant art is dependent on the diagram shown in Figure 1, in which the numbering of various nucleotides in a hammerhead ribozyme is provided.

Odai *et al.*, *FEBS* 1990, 267:150, state that substitution of guanosine (G) at position 5 of a hammerhead ribozyme for inosine greatly reduces catalytic activity, suggesting "the importance of the 2-amino group of this guanosine for catalytic activity."

Fu and McLaughlin, *Proc. Natl. Acad. Sci. (USA)* 1992, 89:3985, state that deletion of the 2-amino group of the guanosine at position 5 of a hammerhead ribozyme, or deletion of either of the 2'-hydroxyl groups at

position 5 or 8, resulted in ribozymes having a decrease in cleavage efficiency.

5 Fu and McLaughlin, *Biochemistry* 1992, 31:10941, state that substitution of 7-deazaadenosine for adenosine residues in a hammerhead ribozyme can cause reduction in cleavage efficiency. They state that the "results suggest that the N⁷-nitrogen of the adenosine (A) at position 6 in the hammerhead ribozyme/substrate complex is critical for efficient cleavage activity." They go on to indicate that there are five critical functional groups located within the tetrameric sequence GAUG in the hammerhead ribozyme.

10 Slim and Gait, 1992, *BBRC* 183, 605, state that the substitution of guanosine at position 12, in the core of a hammerhead ribozyme, with inosine inactivates the ribozyme.

15 Tuschl *et al.*, 1993 *Biochemistry* 32, 11658, state that substitution of guanosine residues at positions 5, 8 and 12, in the catalytic core of a hammerhead, with inosine, 2-aminopurine, xanthosine, isoguanosine or deoxyguanosine cause significant reduction in the catalytic efficiency of a hammerhead ribozyme.

20 Fu *et al.*, 1993 *Biochemistry* 32, 10629, state that deletion of guanine N⁷, guanine N² or the adenine N⁶-nitrogen within the core of a hammerhead ribozyme causes significant reduction in the catalytic efficiency of a hammerhead ribozyme.

25 Grasby *et al.*, 1993 *Nucleic Acids Res.* 21, 4444, state that substitution of guanosine at positions 5, 8 and 12 positions within the core of a hammerhead ribozyme with O⁶-methylguanosine results in an approximately 75-fold reduction in k_{cat}.

Seela *et al.*, 1993 *Helvetica Chimica Acta* 76, 1809, state that substitution of adenine at positions 13, 14 and 15, within the core of a hammerhead ribozyme, with 7-deazaadenosine does not significantly decrease the catalytic efficiency of a hammerhead ribozyme.

Adams *et al.*, 1994 *Tetrahedron Letters* 35, 765, state that substitution of uracil at position 17 within the hammerhead ribozyme-substrate complex with 4-thiouridine results in a reduction in the catalytic efficiency of the ribozyme by 50 percent.

- 5 Ng *et al.*, 1994 *Biochemistry* 33, 12119, state that substitution of adenine at positions 6, 9 and 13 within the catalytic core of a hammerhead ribozyme with isoguanosine, significantly decreases the catalytic activity of the ribozyme.

- 10 Jennings *et al.*, U.S. Patent 5,298,612, indicate that nucleotides within a "minizyme" can be modified. They state-

- 15 "Nucleotides comprise a base, sugar and a monophosphate group. Accordingly, nucleotide derivatives or modifications may be made at the level of the base, sugar or monophosphate groupings..... Bases may be substituted with various groups, such as halogen, hydroxy, amine, alkyl, azido, nitro, phenyl and the like."

- 20 WO93/23569, WO95/06731, WO95/04818, and WO95/133178 describe various modifications that can be introduced into ribozyme structures.

- 25 This invention relates to production of enzymatic RNA molecules or ribozymes having enhanced or reduced binding affinity and enhanced enzymatic activity for their target nucleic acid substrate by inclusion of one or more modified nucleotides in the substrate binding portion of a ribozyme such as a hammerhead, hairpin, VS ribozyme or hepatitis delta virus derived ribozyme. Applicant has recognized that only small changes in the extent of base-pairing or hydrogen bonding between the ribozyme and substrate can have significant effect on the enzymatic activity of the ribozyme on that substrate. Thus, applicant has recognized that a subtle alteration in the extent of hydrogen bonding along a substrate binding arm of a ribozyme can be used to improve the ribozyme activity compared to an unaltered ribozyme containing no such altered nucleotide. Thus, for example, a guanosine base may be replaced with an inosine to produce a weaker interaction between a ribozyme and its substrate, or a uracil may be replaced with a bromouracil (BrU) to increase the hydrogen bonding interaction with an adenosine. Other
- 30
- 35

examples of alterations of the four standard ribonucleotide bases are shown in Figures 22a-d with weaker or stronger hydrogen bonding abilities shown in each figure.

- In addition, applicant has determined that base modification within some catalytic core nucleotides maintains or enhances enzymatic activity compared to an unmodified molecule. Such nucleotides are noted in Figure 23. Specifically, referring to Figure 23, the preferred sequence of a hammerhead ribozyme in a 5' to 3' direction of the catalytic core is CUG ANG A G•C GAA A, wherein N can be any base or may lack a base (abasic); G•C is a base-pair.
- 10 The nature of the base-paired stem II (Figures 1, 2 and 23) and the recognition arms of stems I and III are variable. In this invention, the use of base-modified nucleotides in those regions that maintain or enhance the catalytic activity and/or the nuclease resistance of the hammerhead ribozyme are described. (Bases which can be modified include those shown in capital letters).
- 15 Examples of base-substitutions useful in this invention are shown in Figure 22, 24-30, 39-43, 45-46. In preferred embodiments cytidine residues are substituted with 5-alkylcytidines (e.g., 5-methylcytidine, Figure 24, R=CH₃, 9), and uridine residues with 5-alkyluridines (e.g., ribothymidine (Figure 24, R=CH₃, 4) or 5-halouridine (e.g., 5-bromouridine, Figure 24, X=Br, 13) or 6-azapyrimidines (Figure 24, 17) or 6-alkyluridine (Figure 30). Guanosine or adenosine residues may be replaced by diaminopurine residues (Figure 24, 22) in either the core or stems. In those bases where none of the functional groups are important in the complexing of magnesium or other functions of a ribozyme, they are optionally replaced with a purine ribonucleoside (Figure 24, 23), which significantly reduces the complexity of chemical synthesis of ribozymes, as no base-protecting group is required during chemical incorporation of the purine nucleus. Furthermore, as discussed above, base-modified nucleotides may be used to enhance the specificity or strength of binding of the recognition arms with similar modifications. Base-modified nucleotides, in general, may also be used to enhance the nuclease resistance of the catalytic nucleic acids in which they are incorporated. These modifications within the hammerhead ribozyme motif are meant to be non-limiting example. Those skilled in the art will recognize that other ribozyme

motifs with similar modifications can be readily synthesized and are within the scope of this invention.

Substitutions of sugar moieties as described in the art cited above, may also be made to enhance catalytic activity and/or nuclease stability.

5 The invention provides ribozymes having increased enzymatic activity in vitro and in vivo as can be measured by standard kinetic assays. Thus, the kinetic features of the ribozyme are enhanced by selection of appropriate modified bases in the substrate binding arms. Applicant recognizes that while strong binding to a substrate by a ribozyme enhances specificity, it may also
10 prevent separation of the ribozyme from the cleaved substrate. Thus, applicant provides means by which optimization of the base pairing can be achieved. Specifically, the invention features ribozymes with modified bases with enzymatic activity at least 1.5 fold (preferably 2 or 3 fold) or greater than the unmodified corresponding ribozyme. The invention also features a
15 method for optimizing the kinetic activity of a ribozyme by introduction of modified bases into a ribozyme and screening for those with higher enzymatic activity. Such selection may be in vitro or in vivo. By enhanced activity is meant to include activity measured in vivo where the activity is a reflection of both catalytic activity and ribozyme stability. In this invention, the product of
20 these properties is increased or not significantly (less than 10 fold) decreased in vivo compared to an all RNA ribozyme.

By "enzymatic portion" is meant that part of the ribozyme essential for cleavage of an RNA substrate.

By "substrate binding arm" is meant that portion of a ribozyme which is
25 complementary to (*i.e.*, able to base-pair with) a portion of its substrate. Generally, such complementarity is 100%, but can be less if desired. For example, as few as 10 bases out of 14 may be base-paired. Such arms are shown generally in Figures 1-3 as discussed below. That is, these arms contain sequences within a ribozyme which are intended to bring ribozyme and target RNA together through complementary base-pairing interactions;
30 *e.g.*, ribozyme sequences within stems I and III of a standard hammerhead ribozyme make up the substrate-binding domain (see Figure 1).

By "unmodified nucleotide base" is meant one of the bases adenine, cytosine, guanosine, uracil joined to the 1' carbon of β -D-ribo-furanose. The sugar also has a phosphate bound to the 5' carbon. These nucleotides are bound by a phosphodiester between the 3' carbon of one nucleotide and the 5' carbon of the next nucleotide to form RNA.

By "modified nucleotide base" is meant any nucleotide base which contains a modification in the chemical structure of an unmodified nucleotide base which has an effect on the ability of that base to hydrogen bond with its normal complementary base, either by increasing the strength of the hydrogen bonding or by decreasing it (*e.g.*, as exemplified above for inosine and bromouracil). Other examples of modified bases include those shown in Figures 22a-d and other modifications well known in the art, including heterocyclic derivatives and the like.

In preferred embodiments the modified ribozyme is a hammerhead, hairpin VS ribozyme or hepatitis delta virus derived ribozyme, and the hammerhead ribozyme includes between 32 and 40 nucleotide bases. The selection of modified bases is most preferably chosen to enhance the enzymatic activity (as observed in standard kinetic assays designed to measure the kinetics of cleavage) of the selected ribozyme, *i.e.*, to enhance the rate or extent of cleavage of a substrate by the ribozyme, compared to a ribozyme having an identical nucleotide base sequence without any modified base.

By "kinetic assays" or "kinetics of cleavage" is meant an experiment in which the rate of cleavage of target RNA is determined. Often a series of assays are performed in which the concentrations of either ribozyme or substrate are varied from one assay to the next in order to determine the influence of that parameter on the rate of cleavage.

By "rate of cleavage" is meant a measure of the amount of target RNA cleaved as a function of time.

Enzymatic nucleic acid having a hammerhead configuration and modified bases which maintain or enhance enzymatic activity are provided. Such nucleic acid is also generally more resistant to nucleases than

unmodified nucleic acid. By "modified bases" in this aspect is meant those shown in Figure 22 A-D, and 24, 30, and 42B or their equivalents; such bases may be used within the catalytic core of the enzyme as well as in the substrate-binding regions. In particular, the invention features modified ribozymes having a base substitution selected from pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyluracil, dihydrouracil, naphthyl, 6-methyl-uracil and aminophenyl. As noted above, substitution in the core may decrease in vitro activity but enhances stability. Thus, in vivo the activity may not be significantly lowered. As exemplified herein such ribozymes are useful in vivo even if active over all is reduced 10 fold. Such ribozymes herein are said to "maintain" the enzymatic activity on all RNA ribozyme.

Small scale synthesis were conducted on a 394 Applied Biosystems, Inc. synthesizer using a modified 2.5 μ mol scale protocol with a 5 min coupling step for alkylsilyl protected nucleotides and 2.5 min coupling step for 2'-O-methylated nucleotides. Table CII outlines the amounts, and the contact times, of the reagents used in the synthesis cycle. A 6.5-fold excess (163 μ L of 0.1 M = 16.3 μ mol) of phosphoramidite and a 24-fold excess of S-ethyl tetrazole (238 μ L of 0.25 M = 59.5 μ mol) relative to polymer-bound 5'-hydroxyl was used in each coupling cycle. Average coupling yields on the 394 Applied Biosystems, Inc. synthesizer, determined by colorimetric quantitation of the trityl fractions, were 97.5-99%. Other oligonucleotide synthesis reagents for the 394 Applied Biosystems, Inc. synthesizer: detritylation solution was 2% TCA in methylene chloride (ABI); capping was performed with 16% N-methyl imidazole in THF (ABI) and 10% acetic anhydride/10% 2,6-lutidine in THF (ABI); oxidation solution was 16.9 mM I₂, 49 mM pyridine, 9% water in THF (Millipore). B & J Synthesis Grade acetonitrile was used directly from the reagent bottle. S-Ethyl tetrazole solution (0.25 M in acetonitrile) was made up from the solid obtained from American International Chemical, Inc.

Deprotection of the RNA was performed as follows. The polymer-bound oligoribonucleotide, trityl-off, was transferred from the synthesis column to a 4mL glass screw top vial and suspended in a solution of methylamine (MA) at 65 °C for 10 min. After cooling to -20 °C, the supernatant was removed from the polymer support. The support was washed three times with 1.0 mL of

EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder.

5 The base-deprotected oligoribonucleotide was resuspended in anhydrous TEA•HF/NMP solution (250 µL of a solution of 1.5mL *N*-methylpyrrolidinone, 750 µL TEA and 1.0 mL TEA•3HF to provide a 1.4M HF concentration) and heated to 65°C for 1.5 h. The resulting, fully deprotected, oligomer was quenched with 50 mM TEAB (9 mL) prior to anion exchange desalting.

10 For anion exchange desalting of the deprotected oligomer, the TEAB solution was loaded onto a Qiagen 500® anion exchange cartridge (Qiagen Inc.) that was prewashed with 50 mM TEAB (10 mL). After washing the loaded cartridge with 50 mM TEAB (10 mL), the RNA was eluted with 2 M TEAB (10 mL) and dried down to a white powder.

15 Inactive hammerhead ribozymes were synthesized by substituting a U for G₅ and a U for A₁₄ (numbering from (Hertel, K. J., *et al.*, 1992, *Nucleic Acids Res.*, 20, 3252)).

The average stepwise coupling yields were >98% (Wincott *et al.*, 1995 *Nucleic Acids Res.* 23, 2677-2684).

20 Hairpin ribozymes are synthesized either as one part or in two parts and annealed to reconstruct the active ribozyme (Chowrira and Burke, 1992 *Nucleic Acids Res.*, 20, 2835-2840).

Ribozymes are purified by gel electrophoresis using general methods or are purified by high pressure liquid chromatography (HPLC; See Stinchcomb *et al.*, International PCT Publication No. WO 95/23225, and are resuspended in water.

25 Various modifications to ribozyme structure can be made to enhance the utility of ribozymes. Such modifications will enhance shelf-life, half-life *in vitro*, stability, and ease of introduction of such ribozymes to the target site, *e.g.*, to enhance penetration of cellular membranes, and confer the ability to recognize and bind to targeted cells.

Examples of such ribozymes are provided in Usman et al., WO 95/13378 and below.

2'-deoxy-2'-nucleotides

Eckstein *et al.*, International Publication No. WO 92/07065; Perrault *et al.*, 1990 Nature 344, 565; Pieken *et al.*, 1991 Science 253, 314; Usman and Cedergren, 1992 Trends in Biochem. Sci. 17, 334; Usman *et al.*, International Publication No. WO 93/15187; and Rossi *et al.*, International Publication No. WO 91/03162, as well as Stinchcomb *et al.*, *supra*, Sproat, European Patent Application 92110298.4 and U.S. Patent 5,334,711; Jennings *et al.*, WO 94/13688 and Beigelman *et al.*, *supra* which describe various chemical modifications that can be made to the sugar moieties of enzymatic RNA molecules. Usman *et al.* also describe various required ribonucleotides in a ribozyme, and methods by which such nucleotides can be defined. De Mesmaeker *et al.* Syn. Lett. 1993, 677-680 (not admitted to be prior art to the present invention) describes the synthesis of certain 2'-C-alkyl uridine and thymidine derivatives. They conclude that "...their use in an antisense approach seems to be very limited."

This invention relates to the use of 2'-deoxy-2'-alkylnucleotides in oligonucleotides, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and also as antisense oligonucleotides. As the term is used in this application, 2'-deoxy-2'-alkylnucleotide-containing enzymatic nucleic acids are catalytic nucleic acid molecules that contain 2'-deoxy-2'-alkylnucleotide components replacing, but not limited to, double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

Also within the invention are 2'-deoxy-2'-alkylnucleotides which may be present in enzymatic nucleic acid or even in antisense oligonucleotides. Contrary to the findings of De Mesmaeker *et al.* applicant has found that such

nucleotides are useful since they enhance the stability of the antisense or enzymatic molecule, and can be used in locations which do not affect the desired activity of the molecule. That is, while the presence of the 2'-alkyl group may reduce binding affinity of the oligonucleotide containing this modification, if that moiety is not in an essential base pair forming region then the enhanced stability that it provides to the molecule is advantageous. In addition, while the reduced binding may reduce enzymatic activity, the enhanced stability may make the loss of activity of less consequence. Thus, for example, if a 2'-deoxy-2'-alkyl-containing molecule has 10% the activity of the unmodified molecule, but has 10-fold higher stability *in vivo* then it has utility in the present invention. The same analysis is true for antisense oligonucleotides containing such modifications. The invention also relates to novel intermediates useful in the synthesis of such nucleotides and oligonucleotides (examples of which are shown in the Figures 48-54), and to methods for their synthesis.

Thus, the invention features 2'-deoxy-2'-alkylnucleotides, that is a nucleotide base having at the 2'-position on the sugar molecule an alkyl moiety and in preferred embodiments features those where the nucleotide is not uridine or thymidine. That is, the invention preferably includes all those nucleotides useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed above.

Examples of various alkyl groups useful in this invention are shown in Figure 48, where each R group is any alkyl. These examples are not limiting in the invention. Specifically, an "alkyl" group refers to a saturated aliphatic hydrocarbon, including straight-chain, branched-chain, and cyclic alkyl groups. Preferably, the alkyl group has 1 to 12 carbons. More preferably it is a lower alkyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino, or SH. The term also includes alkenyl groups which are unsaturated hydrocarbon groups containing at least one carbon-carbon double bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkenyl group has 1 to 12 carbons. More preferably it is a lower alkenyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkenyl group may

- be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂, halogen, N(CH₃)₂, amino, or SH. The term "alkyl" also includes alkynyl groups which have an unsaturated hydrocarbon group containing at least one carbon-carbon triple bond, including straight-chain, branched-chain, and cyclic groups. Preferably, the alkynyl group has 1 to 12 carbons. More preferably it is a lower alkynyl of from 1 to 7 carbons, more preferably 1 to 4 carbons. The alkynyl group may be substituted or unsubstituted. When substituted the substituted group(s) is preferably, hydroxyl, cyano, alkoxy, =O, =S, NO₂ or N(CH₃)₂, amino or SH.
- 10 The term "alkyl" does not include alkoxy groups which have an "-O-alkyl" group, where "alkyl" is defined as described above, where the O is adjacent the 2'-position of the sugar molecule.

- Such alkyl groups may also include aryl, alkylaryl, carbocyclic aryl, heterocyclic aryl, amide and ester groups. An "aryl" group refers to an aromatic group which has at least one ring having a conjugated pi electron system and includes carbocyclic aryl, heterocyclic aryl and biaryl groups, all of which may be optionally substituted. The preferred substituent(s) of aryl groups are halogen, trihalomethyl, hydroxyl, SH, OH, cyano, alkoxy, alkyl, alkenyl, alkynyl, and amino groups. An "alkylaryl" group refers to an alkyl group (as described above) covalently joined to an aryl group (as described above). Carbocyclic aryl groups are groups wherein the ring atoms on the aromatic ring are all carbon atoms. The carbon atoms are optionally substituted. Heterocyclic aryl groups are groups having from 1 to 3 heteroatoms as ring atoms in the aromatic ring and the remainder of the ring atoms are carbon atoms. Suitable heteroatoms include oxygen, sulfur, and nitrogen, and include furanyl, thienyl, pyridyl, pyrrolyl, N-lower alkyl pyrrolo, pyrimidyl, pyrazinyl, imidazolyl and the like, all optionally substituted. An "amide" refers to an -C(O)-NH-R, where R is either alkyl, aryl, alkylaryl or hydrogen. An "ester" refers to an -C(O)-OR', where R is either alkyl, aryl, alkylaryl or hydrogen.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-deoxy-2'-alkylnucleotides (preferably not a 2'-alkyl- uridine or thymidine); e.g. enzymatic nucleic acids having a 2'-deoxy-2'-alkylnucleotide; and a method for producing an

enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group. In other related aspects, the invention features 2'-deoxy-2'-alkylnucleotide triphosphates.

- 5 These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 2'-alkyl derivatives of this invention provide enhanced stability to the oligonucleotides containing them. While they may also reduce absolute activity in an *in vitro* assay they will provide enhanced overall activity *in vivo*.

- 10 Below are provided assays to determine which such molecules are useful. Those in the art will recognize that equivalent assays can be readily devised.

In another aspect, the invention features hammerhead motifs having enzymatic activity having ribonucleotides at locations shown in Figure 47 at 5, 6, 8, 12, and 15.1, and having substituted ribonucleotides at other positions in the core and in the substrate binding arms if desired. (The term "core" refers to positions between bases 3 and 14 in Figure 47, and the binding arms correspond to the bases from the 3'-end to base 15.1, and from the 5'-end to base 2). Applicant has found that use of ribonucleotides at these five locations in the core provide a molecule having sufficient enzymatic activity even when modified nucleotides are present at other sites in the motif. Other such combinations of useful ribonucleotides can be determined as described by Usman *et al. supra*.

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2'-O-alkylthioalkyl and 2'-C-alkylthioalkyl containing nucleic acids

- 25 Medina et al., 1988 *Tetrahedron Letters* 29, 3773, describe a method to convert alcohols to methylthiomethyl ethers.

Matteucci et al., 1990 *Tetrahedron Letters*, 31, 2385, report the synthesis of 3'-5'-methylene bond via a methylthiomethyl precursor.

- 30 Veeneman et al., 1990 *Recl. Trav. Chim. Pays-Bas* 109, 449, report the synthesis of 3'-O-methylthiomethyl deoxynucleoside during the synthesis of a dimer containing 3'-5'-methylene bond.

Jones et al., 1993 *J. Org. Chem.* 58, 2983, report the use of 3'-O-methylthiomethyl deoxynucleoside to synthesize a dimer containing a 3'-thioformacetal internucleoside linkages. The paper also describes a method to synthesize phosphoramidites for DNA synthesis.

- 5 Zavgorodny et al., 1991 *Tetrahedron Letters* 32, 7593, describe a method to synthesize a nucleoside containing methylthiomethyl modification.

This invention relates to the incorporation of 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides into nucleic acids, which are particularly useful for enzymatic cleavage of RNA or single-stranded DNA, and
10 also as antisense oligonucleotides.

As the term is used in this application, 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide or non-nucleotide-containing enzymatic nucleic acids are catalytic nucleic molecules that contain 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide or non-nucleotides components replacing one or
15 more bases or regions including, but not limited to, those bases in double stranded stems, single stranded "catalytic core" sequences, single-stranded loops or single-stranded recognition sequences. These molecules are able to cleave (preferably, repeatedly cleave) separate RNA or DNA molecules in a nucleotide base sequence specific manner. Such catalytic nucleic acids can
20 also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript.

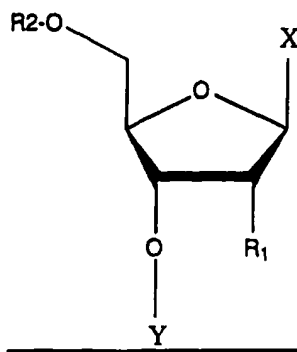
Also within the invention are 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides which may be present in enzymatic nucleic acid or in antisense oligonucleotides or 2-5A antisense chimera. Such
25 nucleotides or non-nucleotides are useful since they enhance the activity of the antisense or enzymatic molecule. The invention also relates to novel intermediates useful in the synthesis of such nucleotides or non-nucleotides and oligonucleotides (examples of which are shown in the Figures), and to methods for their synthesis.

30 Thus, the invention features 2'-O-alkylthioalkyl nucleosides or non-nucleosides, that is a nucleoside or non-nucleosides having at the 2'-position on the sugar molecule a 2'-O-alkylthioalkyl moiety. In a related aspect, the

invention also features 2'-O-alkylthioalkyl nucleotides or non-nucleotides. That is, the invention preferably includes those nucleotides or non-nucleotides having 2' substitutions as noted above useful for making enzymatic nucleic acids or antisense molecules that are not described by the art discussed
5 above.

The term non-nucleotide refers to any group or compound which can be incorporated into a nucleic acid chain in the place of one or more nucleotide units, including either sugar and/or phosphate substitutions, and allows the remaining bases to exhibit their enzymatic activity. The group or compound is
10 abasic in that it does not contain a commonly recognized nucleotide base, such as adenine, guanine, cytosine, uracil or thymine. It may have substitutions for a 2' or 3' H or OH as described in the art. See Eckstein et al. and Usman et al., *supra*.

The term nucleotide refers to the regular nucleotides (A, U, G, T and C)
15 and modified nucleotides such as 6-methyl U, inosine, 5-methyl C and others. Specifically, the term "nucleotide" is used as recognized in the art to include natural bases, and modified bases well known in the art. Such bases are generally located at the 1' position of a sugar moiety. The term "non-nucleotide" as used herein to encompass sugar moieties lacking a base or
20 having other chemical groups in place of a base at the 1' position. Such molecules generally include those having the general formula:



wherein, R1 represents 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl; X represents a base or H; Y represents a phosphorus-containing group; and R2
25 represents H, DMT or a phosphorus-containing group (Figure 55).

Phosphorus-containing group is generally a phosphate, thiophosphate, H-phosphonate, methylphosphonate, phosphoramidite or other modified group known in the art.

In another aspect, the invention features 2'-C-alkylthioalkyl nucleosides or non-nucleosides, that is a nucleotide or a non-nucleotide residue having at the 2'-position on the sugar molecule a 2'-C-alkylthioalkyl moiety. In a related aspect, the invention also features 2'-C-alkylthioalkyl nucleotides or non-nucleotides. That is, the invention preferably includes all those 2' modified nucleotides or non-nucleotides useful for making enzymatic nucleic acids or antisense molecules as described above that are not described by the art discussed above.

Specifically, an "alkyl" group is as defined above, except that the term includes 2'-O-alkyl moieties.

In other aspects, also related to those discussed above, the invention features oligonucleotides having one or more 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides; *e.g.* enzymatic nucleic acids having a 2'-O-methylthiomethyl and/or 2'-C-alkylthioalkyl nucleotides or non-nucleotides; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one nucleotide or a non-nucleotide moiety having at its 2'-position an 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl group.

In other related aspects, the invention features 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl nucleotide triphosphates. These triphosphates can be used in standard protocols to form useful oligonucleotides of this invention.

The 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl derivatives of this invention provide enhanced activity and stability to the oligonucleotides containing them.

In yet another preferred embodiment, the invention features oligonucleotides having one or more 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl abasic (non-nucleotide) moieties. For example, enzymatic

nucleic acids having a 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl abasic moiety; and a method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, by forming the enzymatic molecule with at least one position having at its 2'-
5 position an 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl group.

In related embodiments, the invention features enzymatic nucleic acids containing one or more 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl substitutions either in the enzymatic portion, substrate binding portion or both, as long as the catalytic activity of the ribozyme is not significantly decreased.

10 In yet another preferred embodiment, the invention features the use of 2'-O-alkylthioalkyl moieties as protecting groups for 2'-hydroxyl positions of ribofuranose during nucleic acid synthesis.

While this invention is applicable to all oligonucleotides, applicant has found that the modified molecules of this invention are particularly useful for
15 enzymatic RNA molecules. Thus, below is provided examples of such molecules. Those in the art will recognize that equivalent procedures can be used to make other molecules without such enzymatic activity. Specifically, Figure 1 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided.

20 Referring to Figure 1, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG [base paired with] CGAAA. In this invention, the use of 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl substituted nucleotides or non-nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead
25 ribozyme is described. Substitutions of any nucleotide with any of the modified nucleotides or non-nucleotides discussed above are possible. Usman *et al.*, *supra* and Sproat *et al.*, *supra* as well as other publications indicate those bases that can be substituted in noted ribozyme motifs. Those in the art can thus determine those bases that may be substituted as described
30 herein with beneficial retainment of enzymatic activity and stability.

Non-nucleotides

Usman, et al., WO 93/15187 in discussing modified structures in ribozymes states:

5 It should be understood that the linkages between
the building units of the polymeric chain may be
linkages capable of bridging the units together for
either in vitro or in vivo. For example the linkage
may be a phosphorous containing linkage, e.g.,
10 phosphodiester or phosphothioate, or may be a
nitrogen containing linkage, e.g., amide. It should
further be understood that the chimeric polymer
may contain non-nucleotide spacer molecules
along with its other nucleotide or analogue units.
15 Examples of spacer molecules which may be used
are described in Nielsen et al. Science, 254:1497-
1500 (1991).

Jennings et al., WO 94/13688 while discussing hammerhead ribozymes lacking the usual stem II base-paired region state:

20 One or more ribonucleotides and/or
deoxyribonucleotides of the group (X)_m, [stem II]
may be replaced, for example, with a linker
selected from optionally substituted
polyphosphodiester (such as poly(1-phospho-3-
25 propanol)), optionally substituted alkyl, optionally
substituted polyamide, optionally substituted glycol,
and the like. Optional substituents are well known
in the art, and include alkoxy (such as methoxy,
ethoxy and propoxy), straight or branch chain lower
alkyl such as C₁ - C₅ alkyl), amine, aminoalkyl
30 (such as amino C₁ - C₅ alkyl), halogen (such as F,
C1 and Br) and the like. The nature of optional
substituents is not of importance, as long as the
resultant endonuclease is capable of substrate
cleavage.

35 Additionally, suitable linkers may comprise
polycyclic molecules, such as those containing
phenyl or cyclohexyl rings. The linker (L) may be a
polyether such as polyphosphopropanediol,
polyethyleneglycol, a bifunctional polycyclic
40 molecule such as a bifunctional pentalene, indene,
naphthalene, azulene, heptalene, biphenylene,
asymindacene, sym-indacene, acenaphthylene,
fluorene, phenalene, phenanthrene, anthracene,
fluoranthene, acephenathrylene, aceanthrylene,

5 triphenylene, pyrene, chrysene, naphthacene, thianthrene, isobenzofuran, chromene, xanthene, phenoxathiin, indolizine, isoindole, 3-H-indole, indole, 1-H-indazole, 4-H-quinolizine, isoquinoline, quinoline, phthalazine, naphthyridine, quinoxaline, quinazoline, cinnoline, pteridine, 4- α H-carbazole, carbazole, B-carboline, phenanthridine, acridine, perimidine, phenanthroline, phenazine, phenolthiazine, phenoxazine, which polycyclic
10 compound may be substituted or modified, or a combination of the polyethers and the polycyclic molecules.

The polycyclic molecule may be substituted of polysubstituted with C₁-C₅ alkyl, alkenyl, hydroxyalkyl, halogen or haloalkyl group or with O-A or CH₂-O-A wherein A is H or has the formula CONR'R" wherein R' and R" are the same or different and are hydrogen or a substituted or unsubstituted C₁-C₆ alkyl, aryl, cycloalkyl, or heterocyclic group; or A has the formula -M-NR'R" wherein R' and R" are the same or different and are hydrogen, or a C₁-C₅ alkyl, alkenyl, hydroxyalkyl, or haloalkyl group wherein the halo atom is fluorine, chlorine, bromine, or iodine atom; and -M- is an organic moiety having 1 to 10 carbon atoms and is a branched or straight chain alkyl, aryl, or cycloalkyl group.
15 20 25

In one embodiment, the linker is tetraphosphoropropanediol or pentaphosphoropropanediol. In the case of polycyclic molecules there will be preferably 18 or more atoms bridging the nucleic acids. More preferably their will be from 30 to 50 atoms bridging, see for Example 5. In another embodiment the linker is a bifunctional carbazole or bifunctional carbazole linked to one or more polyphosphoropropanediol.
30 35

Such compounds may also comprise suitable functional groups to allow coupling through reactive groups on nucleotides."
40

This invention concerns the use of non-nucleotide molecules as spacer elements at the base of double-stranded nucleic acid (e.g., RNA or DNA) stems (duplex stems) or more preferably, in the single-stranded regions, catalytic core, loops, or recognition arms of enzymatic nucleic acids. Duplex
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stems are ubiquitous structural elements in enzymatic RNA molecules. To facilitate the synthesis of such stems, which are usually connected via single-stranded nucleotide chains, a base or base-pair mimetic may be used to reduce the nucleotide requirement in the synthesis of such molecules, and to confer nuclease resistance (since they are non-nucleic acid components). This also applies to both the catalytic core and recognition arms of a ribozyme. In particular abasic nucleotides (i.e., moieties lacking a nucleotide base, but having the sugar and phosphate portions) can be used to provide stability within a core of a ribozyme, e.g., at U4 or N7 of a hammerhead structure shown in Figure 1.

Thus, the invention features an enzymatic nucleic acid molecule having one or more non-nucleotide moieties, and having enzymatic activity to cleave an RNA or DNA molecule.

Examples of such non-nucleotide mimetics are shown in Figure 58 and their incorporation into hammerhead ribozymes is shown in Figure 60. These non-nucleotide linkers may be either polyether, polyamine, polyamide, or polyhydrocarbon compounds. Specific examples include those described by Seela and Kaiser, *Nucleic Acids Res.* **1990**, *18*:6353 and *Nucleic Acids Res.* **1987**, *15*:3113; Cload and Schepartz, *J. Am. Chem. Soc.* **1991**, *113*:6324; Richardson and Schepartz, *J. Am. Chem. Soc.* **1991**, *113*:5109; Ma et al., *Nucleic Acids Res.* **1993**, *21*:2585 and *Biochemistry* **1993**, *32*:1751; Durand et al., *Nucleic Acids Res.* **1990**, *18*:6353; McCurdy et al., *Nucleosides & Nucleotides* **1991**, *10*:287; Jäschke et al., *Tetrahedron Lett.* **1993**, *34*:301; Ono et al., *Biochemistry* **1991**, *30*:9914; Arnold et al., International Publication No. WO 89/02439 entitled "Non-nucleotide Linking Reagents for Nucleotide Probes"; and Ferentz and Verdine, *J. Am. Chem. Soc.* **1991**, *113*:4000, all hereby incorporated by reference herein.

In preferred embodiments, the enzymatic nucleic acid includes one or more stretches of RNA, which provide the enzymatic activity of the molecule, linked to the non-nucleotide moiety.

In preferred embodiments, the enzymatic nucleic acid includes one or more stretches of RNA, which provide the enzymatic activity of the molecule,

linked to the non-nucleotide moiety. The necessary ribonucleotide components are known in the art, see, e.g., Usman, supra and Usman et al., Nucl. Acid. Symp. Genes 31:163, 1994.

5 As the term is used in this application, non-nucleotide-containing enzymatic nucleic acid means a nucleic acid molecule that contains at least one non-nucleotide component which replaces a portion of a ribozyme, *e.g.*, but not limited to, a double-stranded stem, a single-stranded "catalytic core" sequence, a single-stranded loop or a single-stranded recognition sequence. These molecules are able to cleave (preferably, repeatedly cleave) separate
10 RNA or DNA molecules in a nucleotide base sequence specific manner. Such molecules can also act to cleave intramolecularly if that is desired. Such enzymatic molecules can be targeted to virtually any RNA transcript. Such molecules also include nucleic acid molecules having a 3' or 5' non-nucleotide, useful as a capping group to prevent exonuclease digestion.

15 Non-nucleotide mimetics useful in this invention are generally described above and in Usman et al. WO 95/06731. Those in the art will recognize that these mimetics can be incorporated into an enzymatic molecule by standard techniques at any desired location. Suitable choices can be made by standard experiments to determine the best location, *e.g.*, by synthesis of the
20 molecule and testing of its enzymatic activity. The optimum molecule will contain the known ribonucleotides needed for enzymatic activity, and will have non-nucleotides which change the structure of the molecule in the least way possible. What is desired is that several nucleotides can be substituted by one non-nucleotide to save synthetic steps in enzymatic molecule synthesis
25 and to provide enhanced stability of the molecule compared to RNA or even DNA.

Synthesis

This invention relates to the synthesis, deprotection, and purification of enzymatic RNA or modified enzymatic RNA molecules in milligram to kilogram
30 quantities with high biological activity. Such syntheses are generally detailed in Stinchcomb et al., WO 95/23225.

This invention relates to the synthesis, deprotection, and purification of enzymatic RNA or modified enzymatic RNA molecules in milligram to kilogram quantities with high biological activity.

Generally, RNA is synthesized and purified by methodologies based on:
5 tetrazole to activate the RNA amidite, NH_4OH to remove the exocyclic amino protecting groups, tetra-*n*-butylammonium fluoride (TBAF) to remove the 2'-OH alkylsilyl protecting groups, and gel purification and analysis of the deprotected RNA. In particular this applies to, but is not limited to, a certain
10 class of RNA molecules, ribozymes. These may be formed either chemically or using enzymatic methods. Examples of the chemical synthesis, deprotection, purification and analysis procedures are provided by Usman et al., 1987 *J. American Chem. Soc.*, 109, 7845, Scaringe et al. *Nucleic Acids Res.* 1990, 18, 5433-5341, Perreault et al. *Biochemistry* 1991, 30 4020-4025, and Slim and Gait *Nucleic Acids Res.* 1991, 19, 1183-1188. Odai et al. *FEBS*
15 *Lett.* 1990, 267, 150-152 describes a reverse phase chromatographic purification of RNA fragments used to form a ribozyme. All the above noted references are all hereby incorporated by reference herein.

The aforementioned chemical synthesis, deprotection, purification and analysis procedures are time consuming (10-15 m coupling times) and may
20 also be affected by inefficient activation of the RNA amidites by tetrazole, time consuming (6-24 h) and incomplete deprotection of the exocyclic amino protecting groups by NH_4OH , time consuming (6-24 h), incomplete and difficult to desalt TBAF-catalyzed removal of the alkylsilyl protecting groups, time consuming and low capacity purification of the RNA by gel
25 electrophoresis, and low resolution analysis of the RNA by gel electrophoresis.

Imazawa and Eckstein, 1979 *J. Org. Chem.*, 12, 2039, describe the synthesis of 2'-amino-2'-deoxyribofuranosyl purines. They state that-

30 "To protect the 2'-amino function, we selected the trifluoroacetyl group which can easily be removed."

Chemical linkage

Jennings et al., US Patent No. 5,298,612 describe the use of non-nucleotides to assemble a hammerhead ribozyme lacking a stem II portion.

Draper et al., WO 93/23569 (PCT/US93/04020) describes synthesis of
5 ribozymes in two parts in order to aid in the synthetic process (see, e.g., p. 40).

Usman et al., WO 95/06731, describe enzymatic nucleic acid molecules having non-nucleotides within their structure. Such non-nucleotides can be used in place of nucleotides to allow formation of an enzymatic nucleic acid.

This invention relates to improved methods for synthesis of enzymatic
10 nucleic acids and, in particular, hammerhead and hairpin motif ribozymes. This invention is advantageous over iterative chemical synthesis of ribozymes since the yield of the final ribozyme can be significantly increased. Rather than synthesizing, for example, a 37mer hammerhead ribozyme, two partial ribozyme portions, e.g., a 20mer and a 17mer, can be synthesized in
15 significantly higher yield, and the two reacted together to form the desired enzymatic nucleic acid.

Referring to Fig. 68, the strategy involved is shown for a hammerhead ribozyme where each n or n' is independently any desired nucleotide or non-nucleotide, each filled-in circle represents pairing between bases or other
20 entities, and the solid line represents a covalent bond. Within the structure each n and n' may be a ribonucleotide, a 2'-methoxy-substituted nucleotide, or any other type of nucleotide which does not significantly affect the desired enzymatic activity of the final product (see Usman et al., supra). In the particular embodiment shown, which is not limiting in this invention, five
25 ribonucleotides are provided at rG5, rA6, rG8, rG12, and rA15.1. U4 and U7 may be abasic (i.e., lacking the uridine moiety) or may be ribonucleotides, 2'-methoxy substituted nucleotides, or other such nucleotides. a9, a13, and a14 are preferably 2'-methoxy or may have other substituents. The synthesis of this hammerhead ribozyme is performed by synthesizing a 3' and a 5' portion
30 as shown in a lower part of Fig. 68. Each 5' and 3' portion has a chemically reactive group X and Y, respectively. Non-limiting examples of such chemically reactive groups are provided in Fig. 69. These groups undergo

chemical reactions to provide the bonds shown in Fig. 69. Thus, the X and Y can be used, in various combinations, in this invention to form a chemical linkage between two ribozyme portions.

Thus, the invention features a method for synthesis of an enzymatically active nucleic acid (as defined by Draper, supra) by providing a 3' and a 5' portion of that nucleic acid, each having independently chemically reactive groups at the 5' and 3' positions, respectively. The reaction is performed under conditions in which a covalent bond is formed between the 3' and 5' portions by those chemically reactive groups. The bond formed can be, but is not limited to, either a disulfide, morpholino, amide, ether, thioether, amine, a double bond, a sulfonamide, carbonate, hydrazone or ester bond. The bond is not the natural bond formed between a 5' phosphate group and a 3' hydroxyl group which is made during normal synthesis of an oligonucleotide. In other embodiments, more than two portions can be linked together using pairs of X and Y groups which allow proper formation of the ribozyme (see Figure 69).

By "chemically reactive group" is simply meant a group which can react with another group to form the desired bonds. These bonds may be formed under any conditions which will not significantly affect the structure of the resulting enzymatic nucleic acid. Those in the art will recognize that suitable protecting groups can be provided on the ribozyme portions.

In preferred embodiments the nucleic acid has a hammerhead motif and the 3' and 5' portions each have chemically reactive groups in or immediately adjacent to the stem II region (see Fig. 1). The stem II region is evident in Fig. 1 between the bases termed a9 and rG12. The C and G within this stem defines the end of the stem II region. Thus, any of the n or n' moieties within the stem II region can be provided with a chemically reactive group. As is evident from this structure, the chemically reactive groups need not be provided in the solid line portion but can be provided at any of the n or n'. In this way the length of each of the 5' and 3' portions can vary by several bases (Figure 70).

In other preferred embodiments, the chemically reactive group can be, but is not limited to, $(CH_2)_nSH$; $(CH_2)_nNHR$; $(CH_2)_nX$; ribose; $COOH$; $(CH_2)_nPPh_3$; $(CH_2)_nSO_2Cl$; $(CH_2)_nCOR$; $(CH_2)_nRNH$ or $(CH_2)_nOH$, where, CH_2 can be replaced by another group which forms a linking chain (which
5 does not interfere with the terminal chemically reactive group) containing various atoms including, but not limited to CH_2 , such as methylenes, ether, ethylene glycol, thioethers, double bonds, aromatic groups and others, generally at most 20 such atoms are provided in the linking chain, most preferably only 5 - 10 atoms, and even more preferably only 3- 5 atoms; each
10 n independently is an integer from 0 to 10 inclusive and may be the same or different; each R independently is a proton or an alkyl, alkenyl (as described above) and other functional groups or conjugates such as peptides, steroids, hormones, lipids, nucleic acid sequences and others that provides nuclease resistance, improved cell association, improved cellular uptake or
15 interacellular localization. X is halogen, and Ph represents a phenyl ring.

In yet other preferred embodiments, the conditions include provision of $NaIO_4$ in contact with the ribose, and subsequent provision of a reducing group such as $NaBH_4$ or $NaCNBH_3$; or the conditions include provision of a coupling reagent.

20 In a second related aspect, the invention features a mixture of the 5' and 3' portions of the enzymatically active nucleic acids having the 3' and 5' chemically reactive groups noted above.

Those in the art will recognize that while examples are provided of half ribozymes it is possible to provide ribozymes in 3 or more portions. For
25 example, the hairpin ribozyme may be synthesized by inclusion of chemically reactive groups in helix IV and in other helices which are not critical to the enzymatic activity of the nucleic acid.

Pol III-based vectors

This invention relates to RNA polymerase III-based methods and systems
30 for expression of therapeutic RNAs in cells *in vivo* or *in vitro*.

The RNA polymerase III (pol III) promoter is one found in DNA encoding 5S, U6, adenovirus VA1, Vault, telomerase RNA, tRNA genes, etc., and is transcribed by RNA polymerase III (for a review see Geiduschek and Tocchini-Valentini, 1988 *Annu. Rev. Biochem.* 57, 873-914; Willis, 1993 *Eur. J. Biochem.* 212, 1-11). There are three major types of pol III promoters: types 1, 2 and 3 (Geiduschek and Tocchini-Valentini, 1988 *supra*; Willis, 1993 *supra*) (see Figure 1). Type 1 pol III promoter consists of three cis-acting sequence elements downstream of the transcriptional start site a) 5' sequence element (A block); b) an intermediate sequence element (I block); c) 3' sequence element (C block). 5S ribosomal RNA genes are transcribed using the type 1 pol III promoter (Specht et al., 1991 *Nucleic Acids Res.* 19, 2189-2191).

The type 2 pol III promoter is characterized by the presence of two cis-acting sequence elements downstream of the transcription start site. All Transfer RNA (tRNA), adenovirus VA RNA and Vault RNA (Kikhofer et al., 1993, *J. Biol. Chem.* 268, 7868-7873) genes are transcribed using this promoter (Geiduschek and Tocchini-Valentini, 1988 *supra*; Willis, 1993 *supra*). The sequence composition and orientation of the two cis-acting sequence elements- A box (5' sequence element) and B box (3' sequence element) are essential for optimal transcription by RNA polymerase III.

The type 3 pol III promoter contains all of the cis-acting promoter elements upstream of the transcription start site. Upstream sequence elements include a traditional TATA box (Mattaj et al., 1988 *Cell* 55, 435-442), proximal sequence element (PSE) and a distal sequence element (DSE; Gupta and Reddy, 1991 *Nucleic Acids Res.* 19, 2073-2075). Examples of genes under the control of the type 3 pol III promoter are U6 small nuclear RNA (U6 snRNA) and Telomerase RNA genes.

In addition to the three predominant types of pol III promoters described above, several other pol III promoter elements have been reported (Willis, 1993 *supra*) (see Figure 76). Epstein-Barr-virus-encoded RNAs (EBER), *Xenopus* seleno-cysteine tRNA and human 7SL RNA are examples of genes that are under the control of pol III promoters distinct from the aforementioned types of promoters. EBER genes contain a functional A and B box (similar to type 2 pol III promoter). In addition they also require an EBER-specific TATA

box and binding sites for ATF transcription factors (Howe and Shu, 1989 *Cell* 57,825-834). The seleno-cysteine tRNA gene contains a TATA box, PSE and DSE (similar to type 3 pol III promoter). Unlike most tRNA genes, the seleno-cysteine tRNA gene lacks a functional A box sequence element. It does
5 require a functional B box (Lee et al., 1989 *J. Biol. Chem.* 264, 9696-9702). The human 7SL RNA gene contains an unique sequence element downstream of the transcriptional start site. Additionally, upstream of the transcriptional start site, the 7SL gene contains binding sites for ATF class of transcription factors and a DSE (Bredow et al., 1989 *Gene* 86, 217-225).

10 Gilboa WO 89/11539 and Gilboa and Sullenger WO 90/13641 describe transformation of eucaryotic cells with DNA under the control of a pol III promoter. They state:

"In an attempt to improve antisense RNA synthesis using stable gene transfer protocols, the use of pol III promoters to drive the expression of antisense RNA can be
15 considered. The underlying rationale for the use of pol III promoters is that they can generate substantially higher levels of RNA transcripts in cells as compared to pol II promoters. For example, it is estimated that in a eucaryotic cell there are about 6×10^7 t-RNA molecules and 7×10^5 mRNA molecules, i.e., about 100 fold more pol III transcripts of this class than total pol II transcripts. Since there are about 100 active t-RNA genes
20 per cell, each t-RNA gene will generate on the average RNA transcripts equal in number to total pol II transcripts. Since an abundant pol II gene transcript represents about 1% of total mRNA while an average pol II transcript represents about 0.01% of total mRNA, a t-RNA (pol III) based transcriptional unit may be able to generate 100 fold to 10,000 fold more RNA than a pol II based transcriptional unit. Several reports have described the
25 use of pol III promoters to express RNA in eucaryotic cells. Lewis and Manley and Sisodia have fused the Adenovirus VA-1 promoter to various DNA sequences (the herpes TK gene, globin and tubulin) and used transfection protocols to transfer the resulting DNA constructs into cultured cells which resulted in transient synthesis of RNA in the transduced cell. De la Pena and Zaslloff have expressed a t-RNA-Herpes TK
30 fusion DNA construct upon microinjection into frog oocytes. Jennings and Molloy have constructed an antisense RNA template by fusing the VA-1 gene promoter to a DNA fragment derived from SV40 based vector which also resulted in transient expression of antisense RNA and limited inhibition of the target gene". [Citations omitted.]

The authors describe a fusion product of a chimeric tRNA and an RNA product (see Fig. 1C of WO 90/13641). In particular they describe a human tRNA^{met} derivative 3-5. 3-5 was derived from a cloned human tRNA gene by deleting 19 nucleotides from the 3' end of the gene. The authors indicate that
5 the truncated gene can be transcribed if a termination signal is provided, but that no processing of the 3' end of the RNA transcript takes place.

Adeniyi-Jones et al., 1984 *Nucleic Acids Res.* 12, 1101-1115, describe certain constructions which "may serve as the basis for utilizing the tRNA gene as a 'portable promoter' in engineered genetic constructions." The authors
10 describe the production of a so-called $\Delta 3'-5$ in which 11 nucleotides of the 3'-end of the mature tRNA^{met} sequence are replaced by a plasmid sequence, and are not processed to generate a mature tRNA. The authors state:

"the properties of the tRNA^{met} 3' deletion plasmids described in this study suggest their potential use in certain engineered genetic constructions. The tRNA gene could
15 be used to promote transcription of theoretically any DNA sequence fused to the 3' border of the gene, generating a fusion gene which would utilize the efficient polymerase III promoter of the human tRNA^{met} gene. By fusion of the DNA sequence to a tRNA^{met} deletion mutant such as $\Delta 3'-4$, a long read-through transcript would be generated in vivo (dependent, of course, on the absence of effective RNA polymerase
20 III termination sequences). Fusion of the DNA sequence to a tRNA^{met} deletion mutant such as $\Delta 3'-5$ would lead to the generation of a co-transcript from which subsequent processing of the tRNA leader at the 5' portion of the fused transcript would be blocked. Control over processing may be of some biological use in engineered constructions, as suggested by properties of mRNA species bearing tRNA sequences as 5' leaders in
25 prokaryotes. Such "dual transcripts" code for several predominant bacterial proteins such as EF-Tu and may use the tRNA leaders as a means of stabilizing the transcript from degradation in vivo. The potential use of the tRNA^{met} gene as a "promoter leader" in eukaryotic systems has been realized recently in our laboratory. Fusion genes consisting of the deleted tRNA^{met} sequences contained on plasmids $\Delta 3'-4$
30 and $\Delta 3'-5$ in front of a promoter-less Herpes simplex type I thymidine kinase gene yield viral-specific enzyme resulting from RNA polymerase III dependent transcription in both *X. laevis* oocytes and somatic cells". [References omitted].

Sullenger et al., 1990 *Cell* 63, 601-619, describe over-expression of *TAR*-containing sequences using a chimeric tRNA^{met}-*TAR* transcription unit in a double copy (DC) murine retroviral vector.

5 Sullenger et al., 1990 *Molecular and Cellular Bio.* 10, 6512, describe expression of chimeric tRNA driven antisense transcripts. It indicates:

10 "successful use of a tRNA-driven antisense RNA transcription system was dependent on the use of a particular type of retroviral vector, the double-copy (DC) vector, in which the chimeric tRNA gene was inserted in the viral LTR. The use of an RNA pol III-based transcription system to stably express high levels of foreign RNA sequences in cells may have other important applications. Foremost, it may significantly improve the ability to inhibit endogenous genes in eucaryotic cells for the study of gene expression and function, whether antisense RNA, ribozymes, or competitors of sequence-specific binding factors are used. tRNA-driven transcription systems may be particularly useful for introducing "mutations" into the germ line, i.e., for generating transgenic animals or transgenic plants. Since tRNA genes are ubiquitously expressed in all cell types, the chimeric tRNA genes may be properly expressed in all tissues of the animal, in contrast to the more idiosyncratic behavior of RNA pol II-based transcription units. However, homologous recombination represents a more elegant although, at present, very cumbersome approach for introducing mutations into the germ line. In either case, the ability to generate transgenic animals or plants carrying defined mutations will be an extremely valuable experimental tool for studying gene function in a developmental context and for generating animal models for human genetic disorders. In addition, tRNA-driven gene inhibition strategies may also be useful in creating pathogen-resistant livestock and plants. [References omitted.]

25 Cotten and Birnstiel, 1989 *EMBO Jnl.* 8, 3861, describe the use of tRNA genes to increase intracellular levels of ribozymes. The authors indicate that the ribozyme coding sequences were placed between the A and the B box internal promoter sequences of the *Xenopus* tRNA^{met} gene. They also indicate that the targeted hammerhead ribozymes were active *in vivo*.

30 Yu et al., 1993 *Proc. Natl. Acad. Sci. USA* 90, 5340, describe the use of a VAI promoter to express a hairpin ribozyme. The resulting transcript consisted

of the first 104 nucleotides of the VAI RNA, followed by the ribozyme sequence and the terminator sequence.

Lieber and Strauss, 1995 *Mol. Cellular Bio.* 15, 540, inserted a hammerhead ribozyme sequence in the central domain of a VAI RNA.

- 5 Pol III-based vectors are described in Stinchcomb et al., WO 95/23225. Another example is provided below.

Example 1: Stromelysin Hammerhead ribozymes

- 10 By engineering ribozyme motifs applicant has designed several ribozymes directed against stromelysin mRNA sequences. These ribozymes are synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave stromelysin target sequences *in vitro* is evaluated.

- 15 The ribozymes are tested for function *in vivo* by analyzing stromelysin expression levels. Ribozymes are delivered to cells by incorporation into liposomes, by complexing with cationic lipids, by microinjection, and/or by expression from DNA/RNA vectors. Stromelysin expression is monitored by biological assays, ELISA, by indirect immunofluorescence, and/or by FACS analysis. Stromelysin mRNA levels are assessed by Northern analysis, RNase protection, primer extension analysis and/or quantitative RT-PCR.
- 20 Ribozymes that block the induction of stromelysin activity and/or stromelysin mRNA by more than 50% are identified.

- 25 Ribozymes targeting selected regions of mRNA associated with arthritic disease are chosen to cleave the target RNA in a manner which preferably inhibits translation of the RNA. Genes are selected such that inhibition of translation will preferably inhibit cell replication, e.g., by inhibiting production of a necessary protein or prevent production of an undesired protein, e.g., stromelysin. Selection of effective target sites within these critical regions of mRNA may entail testing the accessibility of the target RNA to hybridization with various oligonucleotide probes. These studies can be performed using
- 30 RNA or DNA probes and assaying accessibility by cleaving the hybrid molecule with RNaseH (see below). Alternatively, such a study can use

ribozyme probes designed from secondary structure predictions of the mRNAs, and assaying cleavage products by polyacrylamide gel electrophoresis (PAGE), to detect the presence of cleaved and uncleaved molecules.

- 5 In addition, potential ribozyme target sites within the rabbit stromelysin mRNA sequence (1795 nucleotides) were located and aligned with the human target sites. Because the rabbit stromelysin mRNA sequence has an 84% sequence identity with the human sequence, many ribozyme target sites are also homologous. Thus, the rabbit has potential as an appropriate animal
- 10 model in which to test ribozymes that are targeted to human stromelysin but have homologous or nearly homologous cleavage sites on rabbit stromelysin mRNA as well (Tables AII-AVI, AVIII & AIX). Thirty of the 316 UH sites in the rabbit sequence are identical with the corresponding site in the human sequence with respect to at least 14 nucleotides surrounding the potential
- 15 ribozyme cleavage sites. The nucleotide in the RNA substrate that is immediately adjacent (5') to the cleavage site is unpaired in the ribozyme-substrate complex (see Fig. 1) and is consequently not included in the comparison of human and rabbit potential ribozyme sites. In choosing human ribozyme target sites for continued testing, the presence of identical or nearly
- 20 identical sites in the rabbit sequence is considered.

Example 2: Superior sites

- Potential ribozyme target sites were subjected to further analysis using computer folding programs (Mfold or a Macintosh-based version of the following program, LRNA (Zucker (1989) Science 244:48), to determine if 1)
- 25 the target site is substantially single-stranded and therefore predicted to be available for interaction with a ribozyme, 2) if a ribozyme designed to that site is predicted to form stem II but is generally devoid of any other intramolecular base pairing, and 3) if the potential ribozyme and the sequence flanking both sides of the cleavage site together are predicted to interact correctly. The
- 30 sequence of Stem II can be altered to maintain a stem at that position but minimize intramolecular basepairing with the ribozyme's substrate binding arms. Based on these minimal criteria, and including all the sites that are identical in human and rabbit stromelysin mRNA sequence, a subset of 66

potential superior ribozyme target sites was chosen (as first round targets) for continued analysis. These are SEQ. ID. NOS.: 34, 35, 37, 47, 54, 57, 61, 63, 64, 66, 76, 77, 79, 87, 88, 96, 97, 98, 99, 100, 107, 110, 121, 126, 128, 129, 133, 140, 146, 148, 151, 162, 170, 179, 188, 192, 194, 196, 199, 202, 203, 207, 208, 218, 220, 223, 224, 225, 227, 230, 232, 236, 240, 245, 246, 256, 259, 260, 269, 280, 281, 290, 302, 328, 335 and 353 (see Table AIII).

Example 3: Accessible sites

To determine if any or all of these potential superior sites might be accessible to a ribozyme directed to that site, an RNase H assay is carried out. Using this assay, the accessibility of a potential ribozyme target site to a DNA oligonucleotide probe can be assessed without having to synthesize a ribozyme to that particular site. If the complementary DNA oligonucleotide is able to hybridize to the potential ribozyme target site then RNase H, which has the ability to cleave the RNA of a DNA/RNA hybrid, will be able to cleave the target RNA at that particular site. Specific cleavage of the target RNA by RNase H is an indication that that site is "open" or "accessible" to oligonucleotide binding and thus predicts that the site will also be open for ribozyme binding. By comparing the relative amount of specific RNase H cleavage products that are generated for each DNA oligonucleotide/site, potential ribozyme sites can be ranked according to accessibility.

To analyze target sites using the RNase H assay, DNA oligonucleotides (generally 13-15 nucleotides in length) that are complementary to the potential target sites are synthesized. Body-labeled substrate RNAs (either full-length RNAs or ~500-600 nucleotide subfragments of the entire RNA) are prepared by *in vitro* transcription in the presence of a ^{32}P -labeled nucleotide. Unincorporated nucleotides are removed from the ^{32}P -labeled substrate RNA by spin chromatography on a G-50 Sephadex column and used without further purification. To carry out the assay, the ^{32}P -labeled substrate RNA is pre-incubated with the specific DNA oligonucleotide (1 μM and 0.1 μM final concentration) in 20 mM Tris-HCl, pH 7.9, 100 mM KCl, 10 mM MgCl_2 , 0.1 mM EDTA, 0.1 mM DTT at 37°C for 5 minutes. An excess of RNase H (0.8 units/10 μl reaction) is added and the incubation is continued for 10 minutes. The reaction is quenched by the addition of an equal volume of 95% formamide,

20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol FF after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. RNase H-cleaved RNA products are separated from uncleaved RNA on denaturing polyacrylamide gels, visualized
5 by autoradiography and the amount of cleavage product is quantified.

RNase H analysis on the 66 potential ribozyme sites (round 1) was carried out and those DNA oligonucleotides/sites that supported the most RNase H cleavage were determined. These assays were carried out using full-length human and rabbit stromelysin RNA as substrates. Results
10 determined on human stromelysin RNA indicated that 23 of the 66 sites supported a high level of RNase H cleavage, and an additional 13 supported a moderate level of RNase H cleavage. Twenty-two sites were chosen from among these two groups for continued study. Two of the criteria used for making this choice were 1) that the particular site supported at least moderate
15 RNase H cleavage on human stromelysin RNA and 2) that the site have two or fewer nucleotide differences between the rabbit and the human stromelysin sequence. RNase H accessibility on rabbit stromelysin RNA was determined, but was not used as a specific criteria for these choices. Those DNA oligonucleotides that are not totally complementary to the rabbit sequence
20 may not be good indicators of the relative amount of RNase H cleavage, possibly because the mismatch leads to less efficient hybridization of the DNA oligonucleotide to the mismatched RNA substrate and therefore less RNase H cleavage is seen.

Example 4: Analysis of Ribozymes

25 Ribozymes were then synthesized to 22 sites (Table AV) predicted to be accessible as judged the RNase H assay. Eleven of these 22 sites are identical to the corresponding rabbit sites. The 22 sites are SEQ. ID, NOS.: 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, 281. The 22 ribozymes were chemically synthesized
30 with recognition arms of either 7 nucleotides or 8 nucleotides, depending on which ribozyme alone and ribozyme-substrate combinations were predicted by the computer folding program (Mfold) to fold most correctly. After synthesis, ribozymes are either purified by HPLC or gel purified.

These 22 ribozymes were then tested for their ability to cleave both human and rabbit full-length stromelysin RNA. Full-length, body-labeled stromelysin RNA is prepared by *in vitro* transcription in the presence of [α - 32 P]CTP, passed over a G 50 Sephadex column by spin chromatography and used as substrate RNA without further purification. Assays are performed by prewarming a 2X concentration of purified ribozyme in ribozyme cleavage buffer (50 mM Tris-HCl, pH 7.5 at 37°C, 10 mM MgCl₂) and the cleavage reaction is initiated by adding the 2X ribozyme mix to an equal volume of substrate RNA (maximum of 1-5 nM) that has also been prewarmed in cleavage buffer. As an initial screen, assays are carried out for 1 hour at 37°C using a final concentration of 1 μ M and 0.1 μ M ribozyme, *i.e.*, ribozyme excess. The reaction is quenched by the addition of an equal volume of 95% formamide, 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol FF after which the sample is heated to 95°C for 2 minutes, quick chilled and loaded onto a denaturing polyacrylamide gel. Full-length substrate RNA and the specific RNA products generated by ribozyme cleavage are visualized on an autoradiograph of the gel.

Of the 22 ribozymes tested, 21 were able to cleave human and rabbit substrate RNA *in vitro* in a site-specific manner. In all cases, RNA cleavage products of the appropriate lengths were visualized. The size of the RNA was judged by comparison to molecular weight standards electrophoresed in adjacent lanes of the gel. The fraction of substrate RNA cleaved during a ribozyme reaction can be used as an assessment of the activity of that ribozyme *in vitro*. The activity of these 22 ribozymes on full-length substrate RNA ranged from approximately 10% to greater than 95% of the substrate RNA cleaved in the ribozyme cleavage assay using 1 μ M ribozyme as described above. A subset of seven of these ribozymes was chosen for continued study. These seven ribozymes (denoted in Table AV) were among those with the highest activity on both human and rabbit stromelysin RNA. Five of these seven sites have sequence identity between human and rabbit stromelysin RNAs for a minimum of 7 nucleotides in both directions flanking the cleavage site. These sites are 883, 947, 1132, 1221 and 1410. and the ribozymes are SEQ. ID. NOS.: 368, 369, 370, 371, 372, 373, and 374.

Example 5: Arm Length Tests

In order to test the effect of arm length variations on the cleavage activity of a ribozyme to a particular site *in vitro*, ribozymes to these seven sites were designed that had alterations in the binding arm lengths. For each site, a complete set of ribozymes was synthesized that included ribozymes with binding arms of 6 nucleotides, 7 nucleotides, 8 nucleotides, 10 nucleotides and 12 nucleotides, *i.e.*, 5 ribozymes to each site. These ribozymes were gel-purified after synthesis and tested in ribozyme cleavage assays as described above.

After analysis of the 35 ribozymes, five ribozymes with varied arm lengths to each of these seven sites, it was clear that two ribozymes were the most active *in vitro*. These two ribozymes had seven nucleotide arms directed against human sequence cleavage sites of nucleotide 617 and nucleotide 820. These are referred to as RZ 617H 7/7 and RZ 820H 7/7 denoting the human (H) sequence cleavage site (617 or 820) and the arm length on the 5' and 3' side of the ribozyme molecule.

Example 6: Testing the efficacy of ribozymes in cell culture

The two most active ribozymes *in vitro* (RZ 617H 7/7 and RZ 820H 7/7) were then tested for their ability to cleave stromelysin mRNA in the cell. Primary cultures of human or rabbit synovial fibroblasts were used in these experiments. For these efficacy tests, ribozymes with 7 nucleotide arms were synthesized with 2' O- methyl modifications on the 5 nucleotides at the 5' end of the molecule and on the 5 nucleotides at the 3' end of the molecule. For comparison, ribozymes to the same sites but with 12 nucleotide arms (RZ 617H 12/12 and RZ 820H 12/12) were also synthesized with the 2' O methyl modifications at the 5 positions at the end of both binding arms. Inactive ribozymes that contain 2 nucleotide changes in the catalytic core region were also prepared for use as controls. The catalytic core in the inactive ribozymes is C U U A U G A G G C C G A A A G G C C G A U versus C U G A U G A G G C C G A A A G G C C G A A in the active ribozymes. The inactive ribozymes show no cleavage activity *in vitro* when measured on full-length RNA in the typical ribozyme cleavage assay at a 1 μ M concentration for 1 hour.

The general assay was as follows: Fibroblasts, which produce stromelysin, are serum-starved overnight and ribozymes or controls are offered to the cells the next day. Cells are maintained in serum-free media. The ribozyme can be applied to the cells as free ribozyme, or in association with various delivery vehicles such as cationic lipids (including Transfectam™, Lipofectin™ and Lipofectamine™), conventional liposomes, non-phospholipid liposomes or biodegradable polymers. At the time of ribozyme addition, or up to 3 hours later, Interleukin-1 α (typically 20 units/ml) can be added to the cells to induce a large increase in stromelysin expression. The production of stromelysin can then be monitored over a time course, usually up to 24 hours.

If a ribozyme is effective in cleaving stromelysin mRNA within a cell, the amount of stromelysin mRNA will be decreased or eliminated. A decrease in the level of cellular stromelysin mRNA, as well as the appearance of the RNA products generated by ribozyme cleavage of the full-length stromelysin mRNA, can be analyzed by methods such as Northern blot analysis, RNase protection assays and/or primer extension assays. The effect of ribozyme cleavage of cellular stromelysin mRNA on the production of the stromelysin protein can also be measured by a number of assays. These include the ELISA (Enzyme-Linked Immuno Sorbent Assay) and an immunofluorescence assay described below. In addition, functional assays have been published that monitor stromelysin's enzymatic activity by measuring degradation of its primary substrate, proteoglycan.

Example 7: Analysis of Stromelysin Protein

Stromelysin secreted into the media of Interleukin-1 α -induced human synovial fibroblasts was measured by ELISA using an antibody that recognizes human stromelysin. Where present, a Transfectam™-ribozyme complex (0.15 μ M ribozyme final concentration) was offered to $2-4 \times 10^5$ serum-starved cells for 3 hours prior to induction with Interleukin-1 α . The Transfectam™ was prepared according to the manufacturer (Promega Corp.) except that 1:1 (w/w) dioleoyl phosphatidylethanolamine was included. The Transfectam™-ribozyme complex was prepared in a 5:1 charge ratio. Media was harvested 24 hours after the addition of Interleukin-1 α . The control (NO RZ) is Transfectam™ alone applied to the cell. Inactive ribozymes, with 7

- nucleotide arms or 12 nucleotide arms have the two inactivating changes to the catalytic core that are described above. Cell samples were prepared in duplicate and the assay was carried out on several dilutions of the conditioned media from each sample. Results of the ELISA are presented below as a percent of stromelysin present vs. the control (NO RZ) which is set at 100%.

<u>RZ TARGET SITE</u>		
<u>TREATMENT</u>	<u>617H</u>	<u>820H</u>
RZ 7/7	06.83	07.05
RZ 12/12	18.47	33.90
10 INACTIVE RZ 7/7	100	100
INACTIVE RZ 12/12	100	100
NO RZ CONTROL	100	100

- 15 The results above clearly indicate that treatment with active ribozyme, either RZ 617H 7/7 and RZ 820H 7/7, has a dramatic effect on the amount of stromelysin secreted by the cells. When compared to untreated, control cells or cells treated with inactive ribozymes, the level of stromelysin was decreased by approximately 93%. Ribozymes to the same sites, but
- 20 synthesized with 12 nucleotide binding arms, were also efficacious, causing a decrease in stromelysin to ~66 to ~81% of the control. In previous *in vitro* ribozyme cleavage assays, RZ 617H 7/7 and RZ 820H 7/7 had better cleavage activity on full-length RNA substrates than ribozymes with 12 nucleotide arms directed to the same sites (617H 12/12 and RZ 820H 12/12).

25 Example 8: Immunofluorescent Assay

An alternative method of stromelysin detection is to visualize stromelysin protein in the cells by immunofluorescence. For this assay, cells are treated

with monensin to prevent protein secretion from the cell. The stromelysin retained by the cells after monensin addition can then be visualized by immunofluorescence using either conventional or confocal microscopy. Generally, cells were serum-starved overnight and treated with ribozyme the following day for several hours. Monensin was then added and after ~5-6 hours, monensin-treated cells were fixed and permeabilized by standard methods and incubated with an antibody recognizing human stromelysin. Following an additional incubation period with a secondary antibody that is conjugated to a fluorophore, the cells were observed by microscopy. A decrease in the amount of fluorescence in ribozyme-treated cells, compared to cells treated with inactive ribozymes or media alone, indicates that the level of stromelysin protein has been decreased due to ribozyme treatment.

As visualized by the immunofluorescence technique described above, treatment of human synovial fibroblasts with either RZ 617H 7/7 or RZ 820H 7/7 (final concentrations of 1.5 μ M free ribozyme or 0.15 μ M ribozyme complexed with Transfectam™ resulted in a significant decrease in fluorescence, and therefore stromelysin protein, when compared with controls. Controls consisted of treating with media or Transfectam™ alone. Treatment of the cells with the corresponding inactive ribozymes with two inactivating changes in the catalytic core resulted in immunofluorescence similar to the controls without ribozyme treatment.

Rabbit synovial fibroblasts were also treated with RZ 617H 7/7 or RZ 820H 7/7, as well as with the two corresponding ribozymes (RZ 617R 7/7 or RZ 820R 7/7) that each have the appropriate one nucleotide change to make them completely complementary to the rabbit target sequence. Relative to controls that had no ribozyme treatment, immunofluorescence in Interleukin-1 α -induced rabbit synovial fibroblasts was visibly decreased by treatment with these four ribozymes, whether specific for rabbit or human mRNA sequence. For the immunofluorescence study in rabbit synovial fibroblasts, the antibody to human stromelysin was used.

Example 9: Ribozyme Cleavage of Cellular RNA

The following method was used in this example.

Primer extension assay:

The primer extension assay was used to detect full-length RNA as well as the 3' ribozyme cleavage products of the RNA of interest. The method involves synthesizing a DNA primer (generally ~20 nucleotides in length) that can hybridize to a position on the RNA that is downstream (3') of the putative ribozyme cleavage site. Before use, the primer was labeled at the 5' end with ^{32}P [ATP] using T4 polynucleotide kinase and purified from a gel. The labeled primer was then incubated with a population of nucleic acid isolated from a cellular lysate by standard procedures. The reaction buffer was 50 mM Tris-HCl, pH 8.3, 3 mM MgCl_2 , 20 mM KCl, and 10 mM DTT. A 30 minute extension reaction follows, in which all DNA primers that have hybridized to the RNA were substrates for reverse transcriptase, an enzyme that will add nucleotides to the 3' end of the DNA primer using the RNA as a template. Reverse transcriptase was obtained from Life Technologies and is used essentially as suggested by the manufacturer. Optimally, reverse transcriptase will extend the DNA primer, forming cDNA, until the end of the RNA substrate is reached. Thus, for ribozyme-cleaved RNA substrates, the cDNA product will be shorter than the resulting cDNA product of a full-length, or uncleaved RNA substrate. The differences in size of the ^{32}P -labeled cDNAs produced by extension can then be discriminated by electrophoresis on a denaturing polyacrylamide gel and visualized by autoradiography.

Strong secondary structure in the RNA substrate can, however, lead to premature stops by reverse transcriptase. This background of shorter cDNAs is generally not a problem unless one of these prematurely terminated products electrophoreses in the expected position of the ribozyme-cleavage product of interest. Thus, 3' cleavage products are easily identified based on their expected size and their absence from control lanes. Strong stops due to secondary structure in the RNA do, however, cause problems in trying to quantify the total full-length and cleaved RNA present. For this reason, only the relative amount of cleavage can easily be determined.

The primer extension assay was carried out on RNA isolated from cells that had been treated with TransfectamTM-complexed RZ 617H 7/7, RZ 820H 7/7, RZ 617H 12/12 and RZ 820H 12/12. Control cells had been treated with

Transfectam™ alone. Primer extensions on RNA from cells treated with the Transfectam™ complexes of the inactive versions of these four ribozymes were also prepared. The 20 nucleotide primer sequence is 5' AATGAAAACGAGGTCCTTGC 3' and it is complementary to a region about 285 nucleotides downstream of ribozyme site 820. For ribozymes to site 617, the cDNA length for the 3' cleavage product is 488 nucleotides, for 820 the cDNA product is 285 nucleotides. Full-length cDNA will be 1105 nucleotides in length. Where present, 1 ml of 0.15 μ M ribozyme was offered to $\sim 2-3 \times 10^5$ serum-starved human synovial fibroblasts. After 3 hours, 20 units/ml Interleukin-1 α was added to the cells and the incubation continued for 24 hours.

³²P-labeled cDNAs of the correct sizes for the 3' products were clearly visible in lanes that contained RNA from cells that had been treated with active ribozymes to sites 617 and 820. Ribozymes with 7 nucleotide arms were judged to be more active than ribozymes with 12 nucleotide arms by comparison of the relative amount of 3' cleavage product visible. This correlates well with the data obtained by ELISA analysis of the conditioned media from these same samples. In addition, no cDNAs corresponding to the 3' cleavage products were visible following treatment of the cells with any of the inactive ribozymes.

To insure that ribozyme cleavage of the RNA substrate was not occurring during the preparation of the cellular RNA or during the primer extension reaction itself, several controls have been carried out. One control was to add body-labeled stromelysin RNA, prepared by *in vitro* transcription, to the cellular lysate. This lysate was then subjected to the typical RNA preparation and primer extension analysis except that non-radioactive primer was used. If ribozymes that are present in the cell at the time of cell lysis are active under any of the conditions during the subsequent analysis, the added, body-labeled stromelysin RNA will become cleaved. This, however, is not the case. Only full-length RNA was visible by gel analysis, no ribozyme cleavage products were present. This is evidence that the cleavage products detected in RNA from ribozyme-treated cells resulted from ribozyme cleavage in the cell, and not during the subsequent analysis.

Example 10: RNase Protection Assay

By RNase protection analysis, both the 3' and the 5' products generated by ribozyme cleavage of the substrate RNA in a cell can be identified. The RNase protection assay is carried out essentially as described in the protocol provided with the Lysate Ribonuclease Protection Kit (United States Biochemical Corp.) The probe for RNase protection is an RNA that is complementary to the sequence surrounding the ribozyme cleavage site. This "antisense" probe RNA is transcribed *in vitro* from a template prepared by the polymerase chain reaction in which the 5' primer was a DNA oligonucleotide containing the T7 promoter sequence. The probe RNA is body labeled during transcription by including $^{32}\text{P}[\text{CTP}]$ in the reaction and purified away from unincorporated nucleotide triphosphates by chromatography on G-50 Sephadex. The probe RNA (100,000 to 250,000 cpm) is allowed to hybridize overnight at 37°C to the RNA from a cellular lysate or to RNA purified from a cell lysate. After hybridization, RNase T₁ and RNase A are added to degrade all single-stranded RNA and the resulting products are analyzed by gel electrophoresis and autoradiography. By this analysis, full-length, uncleaved target RNA will protect the full-length probe. For ribozyme-cleaved target RNAs, only a portion of the probe will be protected from RNase digestion because the cleavage event has occurred in the region to which the probe binds. This results in two protected probe fragments whose size reflects the position at which ribozyme cleavage occurs and whose sizes add up to the size of the full-length protected probe.

RNase protection analysis was carried out on cellular RNA isolated from rabbit synovial fibroblasts that had been treated either with active or inactive ribozyme. The ribozymes tested had 7 nucleotide arms specific to the rabbit sequence but corresponding to human ribozyme sites 617 and 820 (i.e. RZ 617R 7/7, RZ 820R 7/7). The inactive ribozymes to the same sites also had 7 nucleotide arms and included the two inactivating changes described above. The inactive ribozymes were not active on full-length rabbit stromelysin RNA in a typical 1 hour ribozyme cleavage reaction *in vitro* at a concentration of 1 μM . For all samples, one ml of 0.15 μM ribozyme was administered as a Transfectam™ complex to serum-starved cells. Addition of Interleukin-1 α followed 3 hours later and cells were harvested after 24 hours. For samples

from cells treated with either active ribozyme tested, the appropriately-sized probe fragments representing ribozyme cleavage products were visible. For site 617, two fragments corresponding to 125 and 297 nucleotides were present, for site 820 the two fragments were 328 and 94 nucleotides in length.

- 5 No protected probe fragments representing RNA cleavage products were visible in RNA samples from cells that not been treated with any ribozyme, or in cells that had received the inactive ribozymes. Full-length protected probe (422 nucleotides in length) was however visible, indicating the presence of full-length, uncleaved stromelysin RNA in these samples.

10 Delivery of Free and Transfectam-Complexed Ribozymes to Fibroblasts

- Ribozymes can be delivered to fibroblasts complexed to a cationic lipid or in free form. To deliver free ribozyme, an appropriate dilution of stock ribozyme (final concentration is usually 1.5 μ M) is made in serum-free medium; if a radioactive tracer is to be used (i.e., 32 P), the specific activity of the ribozyme is adjusted to 800-1200 cpm/pmol. To deliver ribozyme complexed with the cationic lipid Transfectam, the lipid is first prepared as a stock solution containing 1/1 (w/w) dioleoylphosphatidylcholine (DOPE). Ribozyme is mixed with the Transfectam/DOPE mixture at a 1/5 (RZ/TF) charge ratio; for a 36-mer ribozyme, this is a 45-fold molar excess of Transfectam (Transfectam has 4 positive charges per molecule). After a 10 min incubation at room temperature, the mixture is diluted and applied to cells, generally at a ribozyme concentration of 0.15 μ M. For 32 P experiments, the specific activity of the ribozyme is the same as for the free ribozyme experiments.

- After 24 hour, about 30% of the offered Transfectam-ribozyme cpm's are cell-associated (in a nuclease-resistant manner). Of this, about 10-15% of the cpm's represent intact ribozyme; this is about 20-25 million ribozymes per cell. For the free ribozyme, about 0.6% of the offered dose is cell-associated after 24 hours. Of this, about 10-15% is intact; this is about 0.6-0.8 million ribozymes per cell.

30 Example 11: *In vitro* cleavage of stromelysin mRNA by HH ribozymes

In order to screen for additional HH ribozyme cleavage sites, ribozymes, targeted against some of the sites listed in example 2 and Table 3, were

synthesized. These ribozymes were extensively modified such that: 5' terminal nucleotides contain phosphorothioate substitutions; except for five ribose residues in the catalytic core, all the other 2'-hydroxyl groups within the ribozyme were substituted with either 2'-O-methyl groups or 2'-C-allyl
5 modifications. The aforementioned modifications are meant to be non-limiting modifications. Those skilled in the art will recognize that other embodiments can be readily generated using the techniques known in the art.

These ribozymes were tested for their ability to cleave RNA substrates *in vitro*. Referring to Fig. 7, *in vitro* RNA cleavage by HH ribozymes targeted to
10 sites 21, 463, 1049, 1366, 1403, 1410 and 1489 (SEQ. ID. NOS. 35, 98, 202, 263, 279, 281 and 292 respectively) was assayed at 37°C. Substrate RNAs were 5' end-labeled using [γ -³²P]ATP and T4 polynucleotide kinase enzyme. In a standard cleavage reaction under "ribozyme excess" conditions, ~1 nM
15 substrate RNA and 40 nM ribozyme were denatured separately by heating to 90°C for 2 min followed by snap cooling on ice for 10 min. The substrate and the ribozyme reaction mixtures were renatured in a buffer containing 50 mM Tris-HCl, pH 7.5 and 10 mM MgCl₂ at 37°C for 10 min. Cleavage reaction was initiated by mixing the ribozyme and the substrate RNA and incubating at
20 37°C. Aliquots of 5 μ l were taken at regular intervals of time and the reaction quenched by mixing with an equal volume of formamide stop mix. The samples were resolved on a 20% polyacrylamide/urea gel.

A plot of percent RNA substrate cleaved as a function of time is shown in Fig. 7. The plot shows that all six HH ribozymes cleaved the target RNA efficiently. Some HH ribozymes were, however, more efficient than others
25 (e.g., 1049HH cleaves faster than 1366HH).

Ribozyme Efficacy Assay in Cultured HS-27 Cells (Used in the Following Examples):

Ribozymes were assayed on either human foreskin fibroblasts(HS-27) cell line or primary human synovial fibroblasts (HSF). All cells were plated the
30 day before the assay in media containing 10% fetal bovine serum in 24 well plates at a density of 5×10^4 cells/well. At 24 hours after plating, the media was removed from the wells and the monolayers were washed with Dulbeccos

phosphate buffered saline (PBS). The cells were serum starved for 24 h by incubating the cells in media containing 0.5% fetal bovine serum (FBS; 1 ml/well). Ribozyme/lipid complexes were prepared as follows: Ribozymes and LipofectAMINE were diluted separately in serum-free DMEM plus 20 mM
5 Hepes pH 7.3 to 2X final concentration, then equal volumes were combined, vortexed and incubated at 37°C for 15 minutes. The charge ratio of LipofectAmine: ribozyme was 3:1. Cells were washed twice with PBS containing Ca^{2+} and Mg^{2+} . Cells were then treated the ribozyme/lipid complexes and incubated at 37°C for 1.5 hours. FBS was then added to a
10 final concentration of 10%. Two hours after FBS addition, the ribozyme containing solution was removed and 0.5 ml DMEM containing 50 u/ml IL-1, 10% FBS, 20 mM Hepes pH 7.3 added. Supernatants were harvested 16 hours after IL-1 induction and assayed for stromelysin expression by ELISA. Polyclonal antibody against Matrix Metalloproteinase 3 (Biogenesis, NH) was
15 used as the detecting antibody and anti-stromelysin monoclonal antibody was used as the capturing antibody in the sandwich ELISA (Maniatis et al., *supra*) to measure stromelysin expression.

Example 12: Ribozyme-Mediated Inhibition of Stromelysin Expression in human fibroblast cells

20 Referring to Figs. 8 through 13, HH ribozymes, targeted to sites 21, 463, 1049, 1366, 1403, 1410 and 1489 within human stromelysin-1 mRNA, were transfected into HS-27 fibroblast or HSF cell line as described above. Catalytically inactive ribozymes that contain 2 nucleotide changes in the catalytic core region were also synthesized for use as controls. The catalytic
25 core in the inactive ribozymes was CUUAUGAGGCCGAAAGGCCGAU versus CUGAUGAGGCCGAAAGGCCGAA in the active ribozymes. The inactive ribozymes show no cleavage activity *in vitro* when measured on full-length RNA in the typical ribozyme cleavage assay at a 1 μM concentration for 1 hour. Levels of stromelysin protein were measured using a sensitive ELISA
30 protocol as described above. + IL-1 in the figures mean that cells were treated with IL-1 to induce the expression of stromelysin expression. -IL-1 means that the cells were not treated. Figs. 8 through 13 show the dramatic reduction in the levels of stromelysin protein expressed in cells that were transfected with active HH ribozymes. This decrease in the level of

stromelysin production is over and above some non-specific inhibition seen in cells that were transfected with catalytically inactive ribozymes. There is on an average a greater than 50% inhibition in stromelysin production (in cells transfected with active HH ribozymes) when compared with control cells that were transfected with inactive ribozymes. These results suggest that the reduction in stromelysin production in HS-27 cells is mediated by sequence-specific cleavage of human stromelysin-1 mRNA by catalytically active HH ribozymes. Reduction in stromelysin protein production in cells transfected with catalytically inactive ribozymes may be due to some "antisense effect" caused by binding of the inactive ribozyme to the target RNA and physically preventing translation.

Example 13: Ribozyme-mediated inhibition of stromelysin expression in Rabbit Knee

In order to extend the ribozyme efficacy in cell culture, applicant has chosen to use rabbit knee as a reasonable animal model to study ribozyme-mediated inhibition of rabbit stromelysin protein expression. Applicant selected a HH ribozyme (1049HH), targeted to site 1049 within human stromelysin-1 mRNA, for animal studies because site 1049 is 100% identical to site 1060 (Tables AIII and AVI) within rabbit stromelysin mRNA. This has enabled applicant to compare the efficacy of the same ribozyme in human as well as in rabbit systems.

Male New Zealand White Rabbits (3-4 Kg) were anaesthetized with ketamine-HCl/xylazine and injected intra-articularly (I.T.) in both knees with 100 µg ribozyme (e.g., SEQ. ID. NO. 202) in 0.5 ml phosphate buffered saline (PBS) or PBS alone (Controls). The IL-1 (human recombinant IL-1 α , 25 ng) was administered I.T., 24 hours following the ribozyme administration. Each rabbit received IL-1 in one knee and PBS alone in the other. The synovium was harvested 6 hours post IL-1 infusion, snap frozen in liquid nitrogen, and stored at -80°C. Total RNA is extracted with TRIzol reagent (GIBCO BRL, Gaithersburg, MD), and was analyzed by Northern-blot analysis and/or RNase-protection assay. Briefly, 0.5 µg cellular RNA was separated on 1.0 % agarose/formaldehyde gel and transferred to Zeta-Probe GT nylon membrane (Bio-Rad, Hercules, CA) by capillary transfer for ~16 hours. The blots were

baked for two hours and then pre-hybridized for 2 hours at 65°C in 10 ml Church hybridization buffer (7 % SDS, 500 mM phosphate, 1 mM EDTA, 1% Bovine Serum Albumin). The blots were hybridized at 65°C for ~16 hours with 10⁶ cpm/ml of full length ³²P-labeled complementary RNA (cRNA) probes to rabbit stromelysin mRNA (cRNA added to the pre-hybridization buffer along with 100 µl 10mg/ml salmon sperm DNA). The blot was rinsed once with 5% SDS, 25 mM phosphate, 1 mM EDTA and 0.5% BSA for 10 min at room temperature. This was followed by two washes (10 min each wash) with the same buffer at 65°C, which was then followed by two washes (10 min each wash) at 65°C with 1% SDS, 25 mM phosphate and 1 mM EDTA. The blot was autoradiographed. The blot was reprobbed with a 100 nt cRNA probe to 18S rRNA as described above. Following autoradiography, the stromelysin expression was quantified on a scanning densitometer, which is followed by normalization of the data to the 18S rRNA band intensities.

As shown in Figs. 14-16, catalytically active 1049HH ribozyme mediates a decrease in the expression of stromelysin expression in rabbit knees. The inhibition appears to be sequence-specific and ranges from 50-70%.

Example 14: Phosphorothioate-substituted Ribozymes inhibit stromelysin expression in Rabbit Knee

Ribozymes containing four phosphorothioate linkages at the 5' termini enhance ribozyme efficacy in mammalian cells. Referring to Fig. 17, applicant has designed and synthesized hammerhead ribozymes targeted to site 1049 within stromelysin RNA, wherein, the ribozymes contain five phosphorothioate linkages at their 5' and 3' termini. Additionally, these ribozymes contain 2'-O-methyl substitutions at 30 nucleotide positions, 2'-C-allyl substitution at U4 position and 2'-OH at five positions (Fig 17A). As described above, these ribozymes were administered to rabbit knees to test for ribozyme efficacy. The 1049 U4-C-allyl P=S active ribozyme shows greater than 50 % reduction in the level of stromelysin RNA in rabbit knee. Catalytically inactive version of the 1049 U4-C-allyl P=S ribozyme shows ~30% reduction in the level of stromelysin RNA.

Referring to Fig. 18, applicant has also designed and synthesized hammerhead ribozymes targeted to three distinct sites within stromelysin RNA, wherein, the ribozymes contain four phosphorothioate linkages at their 5' termini. Additionally, these ribozymes contain 2'-O-methyl substitutions at 29
5 nucleotide positions, 2'-amino substitutions at U4 and U7 positions and 2'-OH at five positions. As described above, these ribozymes were administered to rabbit knees to test for ribozyme efficacy. As shown in Figures 18-21, ribozymes targeted to sites 1049, 1363 and 1366 are all efficacious in rabbit knee. All three ribozymes decreased the level of stromelysin RNA in rabbit
10 knee by about 50 %.

Sequences and chemical modifications described in figures 17 and 18 are meant to be non-limiting examples. Those skilled in the art will recognize that similar embodiments with other ribozymes and ribozymes containing other chemical modifications can be readily generated using techniques
15 known in the art and are within the scope of the present invention.

Applicant has shown that chemical modifications, such as 6-methyl U and abasic (nucleotide containing no base) moieties can be substituted at certain positions within the ribozyme, for example U4 and U7 positions, without significantly effecting the catalytic activity of the ribozyme. Similarly,
20 3'-3' linked abasic inverted ribose moieties can be used to protect the 3' ends of ribozymes in place of an inverted T without effecting the activity of the ribozyme.

B7-1, B7-2, B7-3 and CD40 are attractive ribozyme targets by several criteria. The molecular mechanism of T cell activation is well-established.
25 Efficacy can be tested in well-defined and predictive animal models. The clinical end-point of graft rejection is clear. Since delivery would be *ex vivo*, treatment of the correct cell population would be assured. Finally, the disease condition is serious and current therapies are inadequate. Whereas protein-based therapies would induce anergy against all antigens encountered during
30 the several week treatment period, *ex vivo* ribozyme therapy provides a direct and elegant approach to truly donor-specific anergy.

Similarly, autoimmune diseases and allergies can be prevented or treated by reversing the devastating course of immune response to self-antigens. Specifically, nucleic acids of this inventions can dampen the response to naturally occurring antigens.

5 Example 15: B7-1, B7-2, B7-3 and/or CD40 Hammerhead ribozymes

By engineering ribozyme motifs we have designed several ribozymes directed against B7-1, B7-2, B7-3 and/or CD40 encoded mRNA sequences. These ribozymes were synthesized with modifications that improve their nuclease resistance. The ability of ribozymes to cleave target sequences *in*
10 *vitro* was evaluated.

Several common human cell lines are available that can be induced to express endogenous B7-1, B7-2, B7-3 and/or CD40 . Alternatively, murine splenic cells can be isolated and induced, to express B7-1 or B7-2, with IL-4 or recombinant CD40 ligand. B7-1 and B7-2 can be detected easily with
15 monoclonal antibodies. Use of appropriate fluorescent reagents and fluorescence-activated cell-sorting (FACS) will permit direct quantitation of surface B7-1 and B7-2 on a cell-by-cell basis. Active ribozymes are expected to directly reduce B7-1 or B7-2 expression. Ribozymes targeted to CD40 would prevent induction of B7-2 by CD40 ligand.

20 Several animal models of transplantation are available – Mouse, rat, Porcine model (Fodor et al., 1994, *Proc. Natl. Acad. Sci. USA* 91, 11153); or Baboon (reviewed by Nowak, 1994 *Science* 266, 1148). B7-1, B7-2, B7-3 and/or CD40 protein levels can be measured clinically or experimentally by FACS analysis. B7-1, B7-2, B7-3 and/or CD40 encoded mRNA levels will be
25 assessed by Northern analysis, RNase-protection, primer extension analysis and/or quantitative RT-PCR. Ribozymes that block the induction of B7-1, B7-2, B7-3 and/or CD40 activity and/or B7-1, B7-2, B7-3 and/or CD40 protein encoding mRNAs by more than 20% *in vitro* will be identified.

30 Several animals models of autoimmune disorders are available– allergic encephalomyelitis (EAE) in Lewis rats (Carlson et al., 1993 *Ann. N.Y. Acad. Sci.* 685, 86); animal models of multiple sclerosis (Wekerle et al., 1994 *Ann.*

Neurol. 36, s47) and rheumatoid arthritis (van Laar et al., 1994 Chem. Immunol. 58, 206).

Several animal models of allergy are available and are reviewed by Kemeny and Diaz-Sanchez, 1990, Clin. Exp. Immunol. 82, 423 and Pretolani et al., 1994 Ann. N.Y.Acad. Sci. 725, 247).

RNA ribozymes and/or genes encoding them will be delivered by either free delivery, liposome delivery, cationic lipid delivery, adeno-associated virus vector delivery, adenovirus vector delivery, retrovirus vector delivery or plasmid vector delivery in these animal model experiments (see above). One dose of a ribozyme vector that constitutively expresses the ribozyme or one or more doses of a stable anti-B7-1, B7-2, B7-3 and/or CD40 ribozymes or a transiently expressing ribozyme vector to donor APC, followed by infusion into the recipient may reduce the incidence of graft rejection. Alternatively, graft tissues may be treated as described above prior to transplantation.

Example 16: Synthesis of 6-methyl-uridine phosphoramidite

Referring to Figure 30, the suspension of 6-methyl-uracil (2.77g, 21.96 mmol) in the mixture of hexamethyldisilazane (50mL) and dry pyridine (50mL) was refluxed for three hours. The resulting clear solution of trimethylsilyl derivative of 6-methyl uracyl was evaporated to dryness and coevaporated 2 times with dry toluene to remove traces of pyridine. To the solution of the resulting clear oil, in dry acetonitrile, 1-O-acetyl-2',3',5'-tri-O-benzoyl-b-D-ribose (10.1g, 20 mmol) was added and the reaction mixture was cooled to 0°C. To the above stirred solution, trimethylsilyl trifluoromethanesulfonate (4.35 mL, 24 mmol) was added dropwise and the reaction mixture was stirred for 1.5 h at 0°C and then 1h at room temperature. After that the reaction mixture was diluted with dichloromethane washed with saturated sodium bicarbonate and brine. The organic layer was evaporated and the residue was purified by flash chromatography on silica gel with ethylacetate-hexane (2:1) mixture as an eluent to give 9.5g (83%) of the compound 2 and 0.8g of the corresponding N¹,N³-bis-derivative.

To the cooled (-10°C) solution of the compound (4.2g, 7.36 mmol) in the mixture of pyridine (60 mL) and methanol (10 mL) ice-cooled 2M aqueous

5 solution of sodium hydroxide (16 mL) was added with constant stirring. The reaction mixture was stirred at -10°C for additional 30 minutes and then neutralized to pH 7 with Dowex 50 (Py⁺). The resin was filtered off and washed with a 200 mL mixture of H₂O - Pyridine (4:1). The combined "mother liquor" and the washings were evaporated to dryness and dried by multiple coevaporation with dry pyridine. The residue was redissolved in dry pyridine and then mixed with dimethoxytrityl chloride (2.99g, 8.03 mmol). The reaction mixture was left overnight at room temperature. Reaction was quenched with methanol (25 mL) and the mixture was evaporated. The residue was
10 dissolved in dichloromethane, washed with saturated aqueous sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel using linear gradient of MeOH (2% to 5%) in CH₂Cl₂ as eluent to give 3.4g (83%) of the compound 6.

15 Example 17: Synthesis of 6-methyl-cytidine phosphoramidite

Triethylamine (13.4 ml, 100 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (6.22g, 90 mmol) and phosphorous oxychloride (1.89 ml, 20 mmol) in 50 ml of anhydrous acetonitrile. To the resulting suspension the solution of 2',3',5'-tri-O-Benzoyl-6-methyl uridine
20 (5.7g, 10 mmol) in 30 ml of acetonitrile was added dropwise and the reaction mixture was stirred for 4 hours at room temperature. Then it was concentrated in vacuo to minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was
25 removed in vacuo. The residue was dissolved in 100 ml of 1,4-dioxane and treated with 50 mL of 29% aq NH₄OH overnight. The solvents were removed in vacuo. The residue was dissolved in the mixture of pyridine (60 mL) and methanol (10 mL), cooled to -15°C and ice-cooled 2M aq solution of sodium hydroxide was added under stirring. The reaction mixture was stirred
30 at -10 to -15°C for additional 30 minutes and then neutralized to pH 7 with Dowex 50 (Py⁺). The resin was filtered off and washed with 200 mL of the mixture H₂O - Py (4:1). The combined mother liquor and washings were evaporated to dryness. The residue was crystallized from aq methanol to give 1.6g (62%) of 6-methyl cytidine.

To the solution of 6-methyl cytidine (1.4g, 5.44 mmol) in dry pyridine 3.11 mL of trimethylchlorosilane was added and the reaction mixture was stirred for 2 hours at room temperature. Then acetic anhydride (0.51 mL, 5.44 mmol) was added and the reaction mixture was stirred for additional 3 hours at room temperature. TLC showed disappearance of the starting material and the reaction was quenched with MeOH (20 mL), ice-cooled and treated with water (20 mL, 1 hour). The solvents were removed in vacuo and the residue was dried by four coevaporations with dry pyridine. Finally it was redissolved in dry pyridine and dimethoxytrityl chloride (2.2 g, 6.52 mmol) was added. The reaction mixture was stirred overnight at room temperature and quenched with MeOH (20 mL). The solvents were removed in vacuo. The remaining oil was dissolved in methylene chloride, washed with saturated sodium bicarbonate and brine. The organic layer was separated and evaporated and the residue was purified by flash chromatography on silica gel with the gradient of MeOH in methylene chloride (3% to 5%) to give 2.4 g (74%) of the compound (4).

Example 18: Synthesis of 6-aza-uridine and 6-aza-cytidine

To the solution of 6-aza uridine (5g, 20.39 mmol) in dry pyridine dimethoxytrityl chloride (8.29g, 24.47 mmol) was added and the reaction mixture was left overnight at room temperature. Then it was quenched with methanol (50 mL) and the solvents were removed in vacuo. The remaining oil was dissolved in methylene chloride and washed with saturated aq sodium bicarbonate and brine. The organic layer was separated and evaporated to dryness. The residue was additionally dried by multiple coevaporations with dry pyridine and finally dissolved in dry pyridine. Acetic anhydride (4.43 mL, 46.7 mmol) was added to the above solution and the reaction mixture was left for 3 hours at room temperature. Then it was quenched with methanol and worked-up as above. The residue was purified by flash chromatography on silica gel using mixture of 2% of MeOH in methylene chloride as an eluent to give 9.6g (75%) of the compound.

Triethylamine (23.7 ml, 170.4 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (10.6g, 153.36 mmol) and phosphorous oxychloride (3.22 ml, 34.08 mmol) in 100 ml of anhydrous acetonitrile. To the resulting suspension the solution of 2',3'-di-O-Acetyl-5'-O-Dimethoxytrityl-6-

aza Uridine (7.13g, 11.36 mmol) in 40 ml of acetonitrile was added dropwise and the reaction mixture was stirred for 6 hours at room temperature. Then it was concentrated in vacuo to minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and the solvent was removed in vacuo. The residue was dissolved in 150 ml of 1,4-dioxane and treated with 50 mL of 29% aq NH_4OH for 20 hours at room temperature. The solvents were removed in vacuo. The residue was purified by flash chromatography on silica gel using linear gradient of MeOH (4% to 10%) in methylene chloride as an eluent to give 3.1g (50%) of azacytidine.

To the stirred solution of 5'-O-Dimethoxytrityl-6-aza cytidine (3g, 5.53 mmol) in anhydrous pyridine trimethylchloro silane (2.41 mL, 19 mmol) was added and the reaction mixture was left for 4 hours at room temperature. Then acetic anhydride (0.63 mL, 6.64 mmol) was added and the reaction mixture was stirred for additional 3 hours at room temperature. After that it was quenched with MeOH (15 mL) and the solvents were removed in vacuo. The residue was treated with 1M solution of tetrabutylammonium fluoride in THF (20°, 30 min) and evaporated to dryness.. The remaining oil was dissolved in methylene chloride, washed with saturated aq sodium bicarbonate and water. The separated organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica gel using 4% MeOH in methylene chloride as an eluent to give 2.9g (89.8%) of the compound.

General Procedure for the Introducing of the TBDMS-Group: To the stirred solution of the protected nucleoside in 50 mL of dry THF and pyridine (4 eq) AgNO_3 (2.4 eq) was added. After 10 minutes tert-butyldimethylsilyl chloride (1.5 eq) was added and the reaction mixture was stirred at room temperature for 12 hours. The resulted suspension was filtered into 100 mL of 5% aq NaHCO_3 . The solution was extracted with dichloromethane (2x100 mL). The combined organic layer was washed with brine, dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography on silica gel with hexanes-ethylacetate (3:2) mixture as eluent.

General Procedure for Phosphitylation: To the ice-cooled stirred solution of protected nucleoside (1 mmol) in dry dichloromethane (20 mL) under argon blanket was added dropwise via syringe the premixed solution of N,N-diisopropylethylamine (2.5eq) and 2-cyanoethyl N'-N-diisopropylchlorophosphoramidite (1.2 eq) in dichloromethane (3 mL).
5 Simultaneously via another syringe N-methylimidazole (1 eq) was added and stirring was continued for 2 hours at room temperature. After that the reaction mixture was again ice-cooled and quenched with 15 ml of dry methanol. After 5 min stirring, the mixture was concentrated in vacuo (<40°C) and purified by
10 flash chromatography on silica gel using hexanes-ethylacetate mixture contained 1% triethylamine as an eluent to give corresponding phosphoroamidite as white foam.

Example 19: RNA cleavage activity of HHA ribozyme substituted with 6-methyl-Uridine

15 Hammerhead ribozymes targeted to site A (see Fig. 31) were synthesized using solid-phase synthesis, as described above. U4 position was modified with 6-methyl-uridine.

RNA cleavage assay *in vitro*:

Substrate RNA is 5' end-labeled using [γ -³²P] ATP and T4 polynucleotide
20 kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl
25 and 10 mM MgCl₂. The reaction is initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels.
30 The results are quantified and percentage of target RNA cleaved is plotted as a function of time.

Referring to Fig. 32, hammerhead ribozymes containing 6-methyl-uridine modification at U4 position cleave the target RNA efficiently.

Example 20: RNA cleavage activity of HHB ribozyme substituted with 6-methyl-Uridine

Hammerhead ribozymes targeted to site B (see Fig. 33) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were modified with 6-methyl-uridine.

RNA cleavage reactions were carried out as described above. Referring to Fig. 34, hammerhead ribozymes containing 6-methyl-uridine modification at U4 and U7 positions cleave the target RNA efficiently.

Example 21: RNA cleavage activity of HHC ribozyme substituted with 6-methyl-Uridine

Hammerhead ribozymes targeted to site C (see Fig. 35) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were modified with 6-methyl-uridine.

RNA cleavage reactions were carried out as described above. Referring to Fig. 36, hammerhead ribozymes containing 6-methyl-uridine modification at U4 positions cleave the target RNA efficiently.

Sequences listed in Figure 23, 31, 33, 35, and others and the modifications described in these figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 22: Inhibition of Rat smooth muscle cell proliferation by 6-methyl-U substituted ribozyme HHA.

Hammerhead ribozyme (HHA) is targeted to a unique site (site A) within *c-myc* mRNA. Expression of *c-myc* protein has been shown to be essential for the proliferation of rat smooth muscle cell (Brown *et al.*, 1992 *J. Biol. Chem.* 267, 4625).

The ribozymes that cleaved site A within *c-myc* RNA described above were assayed for their effect on smooth muscle cell proliferation. Rat vascular smooth muscle cells were isolated and cultured as described (Stinchcomb *et al.*, *supra*). HHA ribozymes were complexed with lipids and delivered into rat smooth muscle cells. Serum-starved cells were stimulated as described by Stinchcomb *et al.*, *supra*. Briefly, serum-starved smooth muscle cells were washed twice with PBS, and the RNA/lipid complex was added. The plates were incubated for 4 hours at 37°C. The medium was then removed and DMEM containing 10% FBS, additives and 10 µM bromodeoxyuridine (BrdU) was added. In some wells, FBS was omitted to determine the baseline of unstimulated proliferation. The plates were incubated at 37°C for 20-24 hours, fixed with 0.3% H₂O₂ in 100% methanol, and stained for BrdU incorporation by standard methods. In this procedure, cells that have proliferated and incorporated BrdU stain brown; non-proliferating cells are counter-stained a light purple. Both BrdU positive and BrdU negative cells were counted under the microscope. 300-600 total cells per well were counted. In the following experiments, the percentage of the total cells that have incorporated BrdU (% cell proliferation) is presented. Errors represent the range of duplicate wells. Percent inhibition then is calculated from the % cell proliferation values as follows: % inhibition = 100 - 100 (Ribozyme - 0% serum)/(Control - 0% serum).

Referring to Figure 37, active ribozymes substituted with 6-methyl-U at position 4 of HHA were successful in inhibiting rat smooth muscle cell proliferation. A catalytically inactive ribozyme (inactive HHA), which has two base substitutions within the core (these mutations inactivate a hammerhead ribozyme; Stinchcomb *et al.*, *supra*), does not significantly inhibit rat smooth muscle cell proliferation.

Example 23: Inhibition of stromelysin production in human synovial fibroblast cells by 6-methyl-U substituted ribozyme HHC.

Hammerhead ribozyme (HHC) is targeted to a unique site (site C) within *stromelysin* mRNA.

The general assay was as described (Draper *et al.*, *supra*). Briefly, fibroblasts, which produce stromelysin, are serum-starved overnight and ribozymes or controls are offered to the cells the next day. Cells were maintained in serum-free media. The ribozyme were applied to the cells as free ribozyme, or in association with various delivery vehicles such as cationic

lipids (including Transfectam™, Lipofectin™ and Lipofectamine™), conventional liposomes, non-phospholipid liposomes or biodegradable polymers. At the time of ribozyme addition, or up to 3 hours later, Interleukin-1 α (typically 20 units/ml) can be added to the cells to induce a large increase in stromelysin expression. The production of stromelysin can then be monitored over a time course, usually up to 24 hours.

Supernatants were harvested 16 hours after IL-1 induction and assayed for stromelysin expression by ELISA. Polyclonal antibody against Matrix Metalloproteinase 3 (Biogenesis, NH) was used as the detecting antibody and anti-stromelysin monoclonal antibody was used as the capturing antibody in the sandwich ELISA (Maniatis *et al.*, *supra*) to measure stromelysin expression.

Referring to Figure 38, HHC ribozyme containing 6-methyl-U modification, caused a significant reduction in the level of stromelysin protein production. Catalytically inactive HHC had no significant effect on the protein level.

Example 24: Synthesis of pyridin-2(4)-one nucleoside 3'-phosphoramidites

General procedure for the preparation of 1-(2,3,5-tri-O-benzoyl- β -D-ribofuranosyl)-2(4)-pyridones (3) and (9)

Referring to Figure 39, 2- or 4-hydroxypyridine (1) or (8) (2.09 g, 22 mmol), 1-O-acetyl-2,3,5-tri-O-benzoyl- β -D-ribofuranose (2) (10.08 g, 20 mmol) and BSA (5.5 ml, 22 mmol) were dissolved in dry acetonitrile (100 ml) under argon at 70°C (oil bath) and the mixture stirred for 10 min. Trimethylsilyl trifluoromethanesulfonate (TMSTf) (5.5 ml, 28.5 mmol) was added and the mixture was stirred for an additional hour for 1 or four hours for 8. The mixture was then cooled to room temperature (RT) followed by dilution, with CHCl₃ (200 ml), and extraction, with sat. aq. NaHCO₃ solution. The organic layer was washed with brine, dried (Na₂SO₄) and evaporated to dryness *in vacuo*. The residue was chromatographed on the column of silica gel; 1-5% gradient of methanol in dichloromethane was used for purification of 3 (98% yield) and 2-10% gradient of methanol in dichloromethane for purification of 9 (84% yield).

1-(β -D-Ribofuranosyl)-2(4)-pyridones (4) and (10)

3 or 9 (18 mmol) was dissolved in 0.3M NaOCH₃ (150 ml) and the solution was stirred at RT for 1 hour. The mixture was then neutralized, with Dowex 50WX8 (Py⁺), the ion-exchanger was filtered off and the filtrate was concentrated to a syrup *in vacuo*. The residue was dissolved in water (100 ml) and the solution was washed with chloroform (2 x 50 ml) and ether (2 x 50 ml). The aqueous layer was evaporated to dryness and the residue was then crystallized from ethyl acetate (3.9 g, 91% 4; Niedballa *et al.*, *Nucleic Acid Chemistry*, Part 1, Townsend, L.B. and Tipson, R.S., Ed.; J. Wiley & Sons, Inc.; New York, 1978, p 481-484); 10 (Niedballa and Vorbrüggen, *J. Org. Chem.* 1974, 39, 3668-3671) was crystallized from ethanol (3.6 g, 84%).

1-(2-O-TBDMSi-5-O-DMT- β -D-ribofuranosyl)-2(4)-pyridones

4 or 10 was 5'-O-dimethoxytritylated according to the standard procedure (see *Oligonucleotide Synthesis: A Practical Approach*, M.J. Gait Ed.; IRL Press, Oxford, 1984, p 27) to yield 5 in 76% yield and pyridin-4-one derivative in 67% yield in the form of yellowish foams after silica gel column chromatography (0.5-10% gradient of methanol in dichloromethane). These compounds were treated with *t*-butyldimethylsilyl chloride under the conditions described by Hakimelahi *et al.*, *Can. J. Chem.* 1982, 60, 1106-1113, and the reaction mixtures were purified by the silica gel column chromatography (20-50% gradient of ethyl acetate in hexanes) to enable faster moving 2'-O-TBDMSi isomers (68.5% and 55%, respectively) as colorless foams.

1-[2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl)-*N,N*-diisopropylphosphoramidite]-2(4)-pyridones (7) and (11)

1-(2-O-TBDMS-5-O-DMT- β -D-ribofuranosyl)-2(4)-pyridones were phosphorylated under conditions described by Tuschl *et al.*, *Biochemistry* 1993, 32, 11658-11668, and the products were isolated by silica gel column chromatography using 15-50% gradient of ethyl acetate in hexanes (1% Et₃N) for 7 (89% yield) and dichloromethane (1% Et₃N) for 11 (94% yield).

Phosphoramidites 7 and 11 were incorporated into ribozymes and substrates using the method of synthesis, deprotection, purification and testing

previously described (Wincott *et al.*, 1995 *supra*). The average stepwise coupling yields were ~98 %.

Example 25: Synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-3-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-phenyl- β -D-

5 ribofuranose (8) phosphoramidites

5-*O*-*t*-Butyldiphenylsilyl-2,3-*O*-isopropylidene-1-deoxy-1-phenyl- β -D-ribofuranose (3)

Referring to Figure 40, compound 3 was prepared using the procedure analogous to that described by Czerniecki and Ville, *J. Org. Chem.* 1989, 54, 610-612. Contrary to their result, we succeeded in obtaining the title compound, by
10 using the more acid resistant *t*-butyldiphenylsilyl group for 5-*O*-protection, instead of *t*-butyldimethylsilyl.

1-Deoxy-1-phenyl- β -D-ribofuranose (5)

Compound 3 (1 g, 2.05 mmol) was dissolved in THF (20 ml) and the solution
15 was mixed with 1M TBAF in THF (3 ml, 3 mmol). The reaction mixture was stirred at RT for 30 min followed by evaporation into a syrup. The residue was applied on to a silica gel column and eluted with hexanes followed by 5-70% gradient of ethyl acetate in hexanes. The 5-*O*-desilylated product was obtained as a colorless foam (0.62 g, 88% yield). This material was dissolved in 70% acetic acid and
20 heated at 100°C (oil bath) for 30 min. Evaporation to dryness under reduced pressure and crystallization of the residual syrup from toluene resulted in 5 (0.49 g, 94% yield), mp 120-121°C.

2-*O*-*t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-1-deoxy-1-phenyl- β -D-ribofuranose (7)

Compound 5 (770 mg, 3.66 mmol) was 5-*O*-dimethoxytritylated according to the standard procedure (Oligonucleotide Synthesis: A Practical Approach, M.J. Gait Ed.; IRL Press, Oxford, 1984, p 27) to yield 1.4 g (75% yield) of 5-*O*-dimethoxytrityl derivative as a yellowish foam, following silica gel column chromatography (0.5-2% gradient of methanol in dichloromethane). This material
25 was treated with *t*-butyldimethylsilyl chloride under the conditions described by Hakimelahi *et al.*, *Can. J. Chem.* 1982, 60, 1106-1113, and the reaction mixture
30

was purified by silica gel column chromatography (2-10% gradient of ethyl acetate in hexanes) to afford a slower moving 2'-O-TBDMSi isomer 7 (0.6 g, 35% yield) as a colorless foam. The faster migrating 3'-O-TBDMSi isomer 6 was also isolated (0.55 g, 32% yield).

5 2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-N,N-diisopropylphosphoramidite)-1-deoxy-1-phenyl- β -D-ribofuranose (8)

Compound 7 (0.87 g, 1.39 mmol) was phosphitylated under conditions described by Tuschl *et al.*, *supra* and the product was isolated by silica gel column chromatography using 0.5% ethyl acetate in toluene (1% Et₃N) for elution
10 (0.85 g, 74% yield).

Example 26: Synthesis of pseudouridine, 3-methyluridine and 2,4,6-trimethoxy benzene nucleoside phosphoramidites

Starting with a pseudo uridine, 3-methyluridine or 2,4,6-trimethoxy benzene nucleoside (Gasparutto *et al.*, *Nucleic Acid Res.* 1992 20, 5159-5166; Kalvoda and Farkas, *Nucleic Acid Chemistry*, Part 1, Townsend, L.B. and Tipson, R.S., Ed.;
15 J. Wiley & Sons, Inc.; New York, 1978, p 481-484), phosphoramidites can be prepared by standard protocols described below (Figure 41).

General Procedure for the Introducing of the TBDMS-Group: To the stirred solution of the protected nucleoside in 50 mL of dry THF and pyridine (4 eq)
20 AgNO₃ (2.4 eq) was added. After 10 minutes tert-butyldimethylsilyl chloride (1.5 eq) was added and the reaction mixture was stirred at room temperature for 12 hours. The resulted suspension was filtered into 100 mL of 5% aq NaHCO₃. The solution was extracted with dichloromethane (2x100 mL). The combined organic layer was washed with brine, dried over Na₂SO₄ and evaporated. The residue
25 was purified by flash chromatography on silica gel with hexanes-ethylacetate (3:2) mixture as eluent.

General Procedure for Phosphitylation: To the ice-cooled stirred solution of protected nucleoside (1 mmol) in dry dichloromethane (20 mL) under argon blanket was added dropwise via syringe the premixed solution of N,N-diisopropylethylamine (2.5eq) and 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (1.2 eq) in dichloromethane (3 mL).
30

Simultaneously via another syringe N-methylimidazole (1 eq) was added and stirring was continued for 2 hours at room temperature. After that the reaction mixture was again ice-cooled and quenched with 15 ml of dry methanol. After 5 min stirring, the mixture was concentrated in vacuo (<40°C) and purified by flash chromatography on silica gel using hexanes-ethylacetate mixture contained 1% triethylamine as an eluent to give corresponding phosphoroamidite as white foam.

Pseudouridine, 3-methyluridine or 2,4,6-trimethoxy benzene phosphoramidites were incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. The ribozymes were deprotected using the standard protocol described above with the exception of ribozymes with pseudouridine. Pseudouridine-modified ribozymes were deprotected first by incubation at room temperature, instead of at 55°C, for 24 hours in a mixture of ethanolic ammonia (3:1).

Example 27: Synthesis of dihydrouridine phosphoramidites

Referring to Figure 42, dihydrouridine phosphoramidite was synthesized based on the method described in Chaix *et al.*, 1989 *Nucleic Acid Res.* 17, 7381-7393 with certain improvements:

I. Uridine (1; 10g, 41mmoles) was dissolved in 200 ml distilled water and to the solution 2g of Rh (10% on alumina) was added. The slurry was brought to 60 psi of hydrogen, and hydrogenation was continued for 16hrs. Reaction was monitored by disappearance of UV absorbing material. All of starting material was converted to dihydrouridine (DHU) and tetrahydrouridine (2:1 based on NMR). Tetrahydrouridine was not removed at this step.

II. Dihydrouridine (2; 10g, 41mmoles) was dissolved in 400ml dry pyridine; dimethylaminopyridine (0.244g, 2mmoles), triethylamine (7.93ml, 56mmoles), and dimethoxytritylchloride (16.3g, 48mmoles) were added and stirred under argon overnight. The reaction was quenched with 50ml methanol, extracted with 400ml 5% sodium bicarbonate, and then 400ml brine. The organic phase was dried over sodium sulphate, filtered, and then dried to a foam. 5'-DMT-DHU (3) was purified by silica gel chromatography (dichloromethane with 0.5-5% gradient of methanol; final yield = 9g; 16.4mmoles).

iii. 5'-DMT-DHU (3; 9.0g, 16.4mmoles) was dissolved in 150ml dry THF. Pyridine (4.9ml, 60mmoles) and silver nitrate (3.35g, 19.7mmoles) were added at room temperature and stirred under argon for 10min., then tert.-butyldimethylsilylchloride (tBDMS-Cl; 3.0g, 19.7mmoles) was added and the slurry
5 was stirred under argon overnight. The reaction was filtered over celite into 500ml aqueous 5% sodium bicarbonate and then extracted with 200ml chloroform. The organic phase was washed with 250ml brine, dried over sodium sulfate, and then evaporated to a yellow foam. 2'-tBDMS, 5'-DMT-DHU (5) was purified by silica gel chromatography away from the 3'-tBDMS, 5'-DMT-DHU (4) (hexanes with 10-50%
10 gradient ether; final yield = 5.1g; 7.7mmoles), dried over sodium sulfate, filtered, and then dried to a white powder. The product was kept under high vacuum for 48hrs.

iv. 5'-DMT, 2'-tBDMS-DHU (5; 2.10g, 3.17mmoles) was dissolved in 40ml anhydrous dichloromethane. NN-dimethylaminopyridine (2.21ml, 12.7mmoles), N-methylimidazole (1.27ml, 1.59mmoles), and chloro-diisopropyl-
15 cyanoethylphosphoramidite (1.2ml, 5.22mmoles) were added and the reaction was stirred under argon for 3hrs. The reaction was quenched with 4ml anhydrous methanol and then evaporated to an oil. Final product (6) was purified by silica gel chromatography (dichloromethane with 0-1% ethanol; 1% triethylamine; final
20 yield = 2.2g; 2.5mmoles).

The dihydrouridine was incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. with improvements—nuceloside-oxalyl-polystyrene derivatized support (Alul *et. al.* Nucleic Acids Res., 1991, 19, 1527-1532) was used. The ribozyme containing the dihydrouridine
25 substitution was deprotected using 30% methyl amine in anhydrous ethanol for 15 min. at room temperature and subsequent treatment with *tert*-butyl-ammonium fluoride in anhydrous THF for 24 hrs. at room temperature.

Example 28: Synthesis of 2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-naphthyl- β -D-
30 ribofuranose (7) phosphoramidites

1-Deoxy-1-naphthyl- β -D-ribofuranose (4)

Referring to Figure 45, the title compound was synthesized from naphthalene 1 and tetra-*O*-acetyl- β -D-ribofuranose 2 according to the procedure of Ohruai *et al.* *Agr. Biol. Chem.* 1972, 36, 1651-1653.

5 2-*O*-*t*-Butyldimethylsilyl-5-*O*-dimethoxytrityl-3-*O*-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-naphthyl- β -D-ribofuranose (7)

7 was synthesized in three steps from 4: a) 5'-*O*-dimethoxytritylation using 4,4'-dimethoxytrityl triflate, followed by chromatographic separation of α and β anomer, respectively; b) 2'-*O*-silylation was carried out as described by Hakimelahi *et al.*, 1982 *supra* (32% yield); c) 3'-*O*-phosphitylation was carried out essentially as described by Tuschl *et al.*, 1993 *supra* (85% yield).

This phosphoramidite is incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. The ribozyme containing naphthyl substitution was deprotected using the standard protocol described above.

15 Example 29: Synthesis of 2-*O*-*t*-Butyldimethylsilyl-5-*O*-Dimethoxytrityl-3-*O*-(2-Cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-Deoxy-1-(*p*-Aminophenyl)- β -D-Ribofuranose phosphoramidites

5-*O*-*t*-Butyldiphenylsilyl-2,3-*O*-isopropylidene-1-deoxy-1-(*p*-bromophenyl)- β -D-ribofuranose (3)

20 Referring to Figure 46, 3 was prepared from 4-bromo-1-lithiobenzene and *t*-butyldiphenylsilyl-2,3-*O*-isopropylidene-D-ribo-1,4-lactone using the procedure analogous to that described by Czernecki and Ville, *J. Org. Chem.* 1989, 54, 610-612. Contrary to their result, we succeeded in obtaining the title compound, by using instead of *t*-butyldimethylsilyl the more acid resistant
25 *t*-butyldiphenylsilyl group for 5-*O*-protection.

5-*O*-*t*-Butyldiphenylsilyl-2,3-*O*-isopropylidene-1-deoxy-1-(*p*-aminophenyl)- β -D-ribofuranose (5)

Compound 3 was aminated using liquid ammonia and CuI as described by Piccirilli *et al.* *Helv. Chim. Acta* 1991, 74, 397-406 to give the title
30 compound in 63% yield.

5-O-*t*-Butyldiphenylsilyl-2,3-O-isopropylidene-1-deoxy-1-[p-(N-TFA)aminophenyl]- β -D-ribofuranose (6)

5 (1.2 g, 2.88 mmol) in dry pyridine (20 ml) was treated with trifluoroacetic anhydride (0.5 ml, 3.6 mmol) for 1 hour at 0 °C. The reaction mixture was then quenched with methanol (5 ml) and evaporated to a syrup. The syrup was partitioned between 5% aq. NaHCO₃ and dichloromethane, organic layer was dried (Na₂SO₄) and evaporated to dryness under reduced pressure. This material was used without further purification in the next step.

1-Deoxy-1-[p-(N-TFA)aminophenyl]- β -D-ribofuranose (7)

10 The title compound was prepared from 6 in an identical manner as for the synthesis of deblocked phenyl analog; (82% overall yield for 5'-O-desilylation and the cleavage of 2',3'-O-isopropylidene group).

2-O-*t*-Butyldimethylsilyl-5-O-dimethoxytrityl-3-O-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite)-1-deoxy-1-[p-(N-TFA)aminophenyl]- β -D-ribofuranose (10)

Using the same three step sequence as for the phenyl analog, 10 was prepared from 7 in 32% overall yield.

20 This phosphoramidite is incorporated into ribozymes using solid phase synthesis as described by Wincott *et al.*, 1995 *supra*. The ribozyme containing aminophenyl substitution was deprotected using the standard protocol described above.

Example 30: RNA cleavage reactions catalyzed by HH-B substituted with modified bases

25 Hammerhead ribozymes targeted to site B (see Fig. 43A) were synthesized using solid-phase synthesis, as described above. U4 and U7 positions were substituted with various base-modifications shown in Figure 43B.

30 RNA cleavage reactions were carried out as described above. Referring to Fig. 43B, hammerhead ribozymes containing base modifications at positions 4 or 7 cleave the target RNA to varying degrees of efficiency. Some of the base modifications at position 7 appear to enhance the catalytic efficiency of the

hammerhead ribozymes compared to a standard base at that position (see Figure 43B, pyridin-4-one, phenyl and 3-methyl U modifications).

5 HH-B ribozymes with either pyridin-4-one or phenyl substitution at position 7 were further characterized (Figure 44). It appears that HH-B ribozyme with pyridin-4-one modification at position 7 cleaves RNA with a 10 fold higher k_{cat} when compared to a ribozyme with a U at position 7 (compare Figure 44 A with 44 B). HH-B ribozyme with a phenyl group at position 7 cleaves RNA with a 3 fold higher k_{cat} when compared to a hammerhead ribozyme with U at position 7 (see Figure 44C).

10 Sequences listed in Figure 23, 31, 33, 35, 43 and the modifications described in these figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other 2'-hydroxyl group modifications, including but not limited to amino acids, peptides
15 and cholesterol, can be readily generated using techniques known in the art, and are within the scope of the present invention.

Example 31: 2'-deoxy-2'-alkylnucleotides

20 Table D2 is a summary of specified catalytic parameters (t_A and t_S) on short substrates *in vitro*, and stabilities of the noted modified catalytic nucleic acids in human serum. U4 and U7 refer to the uracil bases noted in Figure 1. Modifications at the 2'-position are shown in the table.

Table D2

Entry	Modification	$t_{1/2}$ (m) Activity (t_A)	$t_{1/2}$ (m) Stability (t_S)	$\beta = t_S/t_A$ $\times 10$
1	U4 & U7 = U	1	0.1	1
2	U4 & U7 = 2'-O-Me-U	4	260	650
3	U4 = 2'=CH ₂ -U	6.5	120	180
4	U7 = 2'=CH ₂ -U	8	280	350
5	U4 & U7 = 2'=CH ₂ -U	9.5	120	130
6	U4 = 2'=CF ₂ -U	5	320	640
7	U7 = 2'=CF ₂ -U	4	220	550
8	U4 & U7 = 2'=CF ₂ -U	20	320	160
9	U4 = 2'-F-U	4	320	800
10	U7 = 2'-F-U	8	400	500
11	U4 & U7 = 2'-F-U	4	300	750
12	U4 = 2'-C-Allyl-U	3	>500	>1700
13	U7 = 2'-C-Allyl-U	3	220	730
14	U4 & U7 = 2'-C-Allyl-U	3	120	400
15	U4 = 2'-araF-U	5	>500	>1000
16	U7 = 2'-araF-U	4	350	875
17	U4 & U7 = 2'-araF-U	15	500	330
18	U4 = 2'-NH ₂ -U	10	500	500
19	U7 = 2'-NH ₂ -U	5	500	1000
20	U4 & U7 = 2'-NH ₂ -U	2	300	1500
21	U4 = dU	6	100	170
22	U4 & U7 = dU	4	240	600

Figure 47 shows base numbering of a hammerhead motif in which the numbering of various nucleotides in a hammerhead ribozyme is provided. Referring to Figure 47, the preferred sequence of a hammerhead ribozyme in a 5'- to 3'-direction of the catalytic core is CUGANGAG[base paired with]CGAAA. In this invention, the use of 2'-C-alkyl substituted nucleotides that maintain or enhance the catalytic activity and or nuclease resistance of the hammerhead ribozyme is described. Although substitutions of any nucleotide with any of the modified nucleotides shown in Figure 48 are possible, and were indeed synthesized, the basic structure composed of primarily 2'-O-Me nucleotides with selected substitutions was chosen to maintain maximal catalytic activity (Yang *et al.* *Biochemistry* 1992, 31, 5005-5009 and Paoletta *et al.* *EMBO J.* 1992, 11, 1913-1919) and ease of synthesis, but is not limiting to this invention.

Ribozymes from Figure 47 and Table D2 were synthesized and assayed for catalytic activity and nuclease resistance. With the exception of entries 8 and 17, all of the modified ribozymes retained at least 1/10 of the wild-type catalytic activity. From Table D2, all 2'-modified ribozymes showed very large and significant increases in stability in human serum (shown) and in the other fluids described below (Example 3, data not shown). The order of most aggressive nuclease activity was fetal bovine serum > human serum > human plasma > human synovial fluid. As an overall measure of the effect of these 2'-substitutions on stability and activity, a ratio β was calculated (Table D2). This β value indicated that all modified ribozymes tested had significant, >100 - >1700 fold, increases in overall stability and activity. These increases in β indicate that the lifetime of these modified ribozymes *in vivo* are significantly increased which should lead to a more pronounced biological effect.

More general substitutions of the 2'-modified nucleotides from Figure 48 also increased the $t_{1/2}$ of the resulting modified ribozymes. However the catalytic activity of these ribozymes was decreased > 10-fold.

In Figure 53 compound 37 may be used as a general intermediate to prepare derivatized 2'-C-alkyl phosphoramidites, where X is CH₃, or an alkyl, or other group described above.

The following are other non-limiting examples showing the synthesis of nucleic acids using 2'-C-alkyl substituted phosphoramidites, the syntheses of the amidites, their testing for enzymatic activity and nuclease resistance. These examples are diagrammed in Figs 48-54.

5 Example 32: Synthesis of Hammerhead Ribozymes Containing 2'-Deoxy-2'-Alkyl nucleotides & Other 2'-Modified Nucleotides

The method of synthesis used generally follows the procedure for normal RNA synthesis as described in Usman, N.; Ogilvie, K.K.; Jiang, M.-Y.; Cedergren, R.J. *J. Am. Chem. Soc.* **1987**, *109*, 7845-7854 and in
10 Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* **1990**, *18*, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end (compounds 10, 12, 17, 22, 31, 18, 26, 32, 36 and 38). Other 2'-modified phosphoramidites were prepared according to: 3 & 4, Eckstein *et al.*
15 *International Publication No.* WO 92/07065; and 5 Kois *et al.* *Nucleosides & Nucleotides* **1993**, *12*, 1093-1109. The average stepwise coupling yields were ~98%. The 2'-substituted phosphoramidites were incorporated into hammerhead ribozymes as shown in Figure 5. However, these 2'-alkyl substituted phosphoramidites may be incorporated not only into hammerhead
20 ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 33: Ribozyme Activity Assay

Purified 5'-end labeled RNA substrates (15-25-mers) and purified 5'-end
25 labeled ribozymes (~36-mers) were both heated to 95 °C, quenched on ice and equilibrated at 37 °C, separately. Ribozyme stock solutions were 1 mM, 200 nM, 40 nM or 8 nM and the final substrate RNA concentrations were ~ 1 nM. Total reaction volumes were 50 µL. The assay buffer was 50 mM Tris-Cl, pH 7.5 and 10 mM MgCl₂. Reactions were initiated by mixing substrate and
30 ribozyme solutions at t = 0. Aliquots of 5 µL were removed at time points of 1, 5, 15, 30, 60 and 120 m. Each time point was quenched in formamide loading buffer and loaded onto a 15% denaturing polyacrylamide gel for analysis.

Quantitative analyses were performed using a phosphorimager (Molecular Dynamics).

Example 34: Stability Assay

500 pmol of gel-purified 5'-end-labeled ribozymes were precipitated in ethanol and pelleted by centrifugation. Each pellet was resuspended in 20 mL of appropriate fluid (human serum, human plasma, human synovial fluid or fetal bovine serum) by vortexing for 20 s at room temperature. The samples were placed into a 37 °C incubator and 2 mL aliquots were withdrawn after incubation for 0, 15, 30, 45, 60, 120, 240 and 480 m. Aliquots were added to 20 mL of a solution containing 95% formamide and 0.5X TBE (50 mM Tris, 50 mM borate, 1 mM EDTA) to quench further nuclease activity and the samples were frozen until loading onto gels. Ribozymes were size-fractionated by electrophoresis in 20% acrylamide/8M urea gels. The amount of intact ribozyme at each time point was quantified by scanning the bands with a phosphorimager (Molecular Dynamics) and the half-life of each ribozyme in the fluids was determined by plotting the percent intact ribozyme vs the time of incubation and extrapolation from the graph.

Example 35: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-O-Phenoxythio-carbonyl-Uridine (7)

To a stirred solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-uridine, 6, (15.1 g, 31 mmol, synthesized according to *Nucleic Acid Chemistry*, ed. Leroy Townsend, 1986 pp. 229-231) and dimethylaminopyridine (7.57 g, 62 mmol) a solution of phenylchlorothionoformate (5.15 mL, 37.2 mmol) in 50 mL of acetonitrile was added dropwise and the reaction stirred for 8 h. TLC (EtOAc:hexanes / 1:1) showed disappearance of the starting material. The reaction mixture was evaporated, the residue dissolved in chloroform, washed with water and brine, the organic layer was dried over sodium sulfate, filtered and evaporated to dryness. The residue was purified by flash chromatography on silica gel with EtOAc:hexanes / 2:1 as eluent to give 16.44 g (85%) of 7.

Example 36: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl -Uridine (8)

To a refluxing, under argon, solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-2'-O-phenoxythiocarbonyl-uridine, 7, (5 g, 8.03 mmol) and allyltributyltin (12.3 mL, 40.15 mmol) in dry toluene, benzoyl peroxide (0.5 g)
5 was added portionwise during 1 h. The resulting mixture was allowed to reflux under argon for an additional 7-8 h. The reaction was then evaporated and the product 8 purified by flash chromatography on silica gel with EtOAc:hexanes / 1:3 as eluent. Yield 2.82 g (68.7%).

Example 37: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine (9)

10 A solution of 8 (1.25 g, 2.45 mmol) in 10 mL of dry tetrahydrofuran (THF) was treated with a 1 M solution of tetrabutylammoniumfluoride in THF (3.7 mL) for 10 m at room temperature. The resulting mixture was evaporated, the residue was loaded onto a silica gel column, washed with 1 L of chloroform, and the desired deprotected compound was eluted with chloroform:methanol /
15 9:1. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The reaction was quenched with methanol (20 mL), evaporated,
20 dissolved in chloroform, washed with 5% aq. sodium bicarbonate and brine. The organic layer was dried over sodium sulfate and evaporated. The residue was purified by flash chromatography on silica gel, EtOAc:hexanes / 1:1 as eluent, to give 0.85 g (57%) of 9 as a white foam.

Example 38: 5'-O-Dimethoxytrityl-2'-C-Allyl-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (10)

25 5'-O-Dimethoxytrityl-2'-C-allyl-uridine (0.64 g, 1.12 mmol) was dissolved in dry dichloromethane under dry argon. N,N-Diisopropylethylamine (0.39 mL, 2.24 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.35 mL, 1.57 mmol) was added dropwise to
30 the stirred reaction solution and stirring was continued for 2 h at RT. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was concentrated *in vacuo* (40 °C)

and purified by flash chromatography on silica gel using a gradient of 10-60% EtOAc in hexanes containing 1% triethylamine mixture as eluent. Yield: 0.78 g (90%), white foam.

5 Example 39: 3',5'-O-(Tetraisopropyl-disiloxane-1,3-diyl)-2'-C-Allyl-N⁴-Acetyl-Cytidine (11)

Triethylamine (6.35 mL, 45.55 mmol) was added dropwise to a stirred ice-cooled mixture of 1,2,4-triazole (5.66 g, 81.99 mmol) and phosphorous oxychloride (0.86 mL, 9.11 mmol) in 50 mL of anhydrous acetonitrile. To the resulting suspension a solution of 3',5'-O-(tetraisopropyl-disiloxane-1,3-diyl)-
10 2'-C-allyl uridine (2.32 g, 4.55 mmol) in 30 mL of acetonitrile was added dropwise and the reaction mixture was stirred for 4 h at room temperature. The reaction was concentrated *in vacuo* to a minimal volume (not to dryness). The residue was dissolved in chloroform and washed with water, saturated aq. sodium bicarbonate and brine. The organic layer was dried over sodium
15 sulfate and the solvent was removed *in vacuo*. The resulting foam was dissolved in 50 mL of 1,4-dioxane and treated with 29% aq. NH₄OH overnight at room temperature. TLC (chloroform:methanol / 9:1) showed complete conversion of the starting material. The solution was evaporated, dried by coevaporation with anhydrous pyridine and acetylated with acetic anhydride
20 (0.52 mL, 5.46 mmol) in pyridine overnight. The reaction mixture was quenched with methanol, evaporated, the residue was dissolved in chloroform, washed with sodium bicarbonate and brine. The organic layer was dried over sodium sulfate, evaporated to dryness and purified by flash chromatography on silica gel (3% MeOH in chloroform). Yield 2.3 g (90%) as
25 a white foam.

Example 40: 5'-O-Dimethoxytrityl-2'-C-Allyl-N⁴-Acetyl-Cytidine

This compound was obtained analogously to the uridine derivative 9 in 55% yield.

Example 41: 5'-O-Dimethoxytrityl-2'-C-allyl-N⁴-Acetyl-Cytidine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (12)

2'-O-Dimethoxytrityl-2'-C-allyl-N⁴-acetyl cytidine (0.8 g, 1.31 mmol) was dissolved in dry dichloromethane under argon. N,N-Diisopropylethylamine
5 (0.46 mL, 2.62 mmol) was added and the solution was ice-cooled. 2-Cyanoethyl N,N-diisopropylchlorophosphoramidite (0.38 mL, 1.7 mmol) was added dropwise to a stirred reaction solution and stirring was continued for 2 h at room temperature. The reaction mixture was then ice-cooled and quenched with 12 mL of dry methanol. After stirring for 5 m, the mixture was
10 concentrated *in vacuo* (40 °C) and purified by flash chromatography on silica gel using chloroform:ethanol / 98:2 with 2% triethylamine mixture as eluent. Yield: 0.91 g (85%), white foam.

Example 42: 2'-Deoxy-2'-Methylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine
15 14 (Hansske,F.; Madej,D.; Robins,M. J. *Tetrahedron* 1984, 40, 125 and Matsuda,A.; Takenuki,K.; Tanaka,S.; Sasaki,T.; Ueda,T. *J. Med. Chem.* 1991, 34, 812) (2.2 g, 4.55 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column.
20 2'-Deoxy-2'-methylene-uridine (1.0 g, 3.3 mmol, 72.5%) was eluted with 20% MeOH in CH₂Cl₂.

Example 43: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine (15)

2'-Deoxy-2'-methylene-uridine (0.91 g, 3.79 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added
25 dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column
30 using EtOAc:hexanes as eluant to yield 15 (0.43 g, 0.79 mmol, 22%).

Example 44: 5'-O-DMT-2'-Deoxy-2'-Methylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (17)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl- β -D-ribofuranosyl)-uracil (0.43 g, 0.8 mmol) dissolved in dry CH_2Cl_2 (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.28 mL, 1.6 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.25 mL, 1.12 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.3 g, 0.4 mmol, 50%) was purified by flash column chromatography over silica gel using a 25-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.42 (CH_2Cl_2 : MeOH / 15:1)

Example 45: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-Uridine

2'-Keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)uridine 14 (1.92 g, 12.6 mmol) and triphenylphosphine (2.5 g, 9.25 mmol) were dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH_2Cl_2 and chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol, 70%) eluted with 25% hexanes in EtOAc.

Example 46: 2'-Deoxy-2'-Difluoromethylene-Uridine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine (3.1 g, 5.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol, 68%) was eluted with 20% MeOH in CH_2Cl_2 .

Example 47: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine (16)

2'-Deoxy-2'-difluoromethylene-uridine (1.1 g, 4.0 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.42 g, 4.18 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 40% EtOAc:hexanes as eluant to yield 5'-O-DMT-2'-deoxy-2'-difluoromethylene-uridine **16** (1.05 g, 1.8 mmol, 45%).

Example 48: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-Uridine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (18)

1-(2'-Deoxy-2'-difluoromethylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uracil (0.577 g, 1 mmol) dissolved in dry CH₂Cl₂ (15 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.36 mL, 2 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.44 mL, 1.4 mmol). The reaction mixture was stirred for 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product (0.404 g, 0.52 mmol, 52%) was purified by flash chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂: MeOH / 15:1).

Example 49: 2'-Deoxy-2'-Methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-4-N-Acetyl-Cytidine **20**

Triethylamine (4.8 mL, 34 mmol) was added to a solution of POCl₃ (0.65 mL, 6.8 mmol) and 1,2,4-triazole (2.1 g, 30.6 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) uridine **19** (1.65 g, 3.4 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at room temperature for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved in dioxane (10 mL) and aq.

ammonia (20 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (3 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO₃ (5 mL). The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine **20** (1.3 g, 2.5 mmol, 73%) was eluted with 20% EtOAc in hexanes.

10 Example 50: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl-β-D-ribofurano-
syl)-4-N-Acetyl-Cytosine **21**

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine **20** (1.3 g, 2.5 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on silica gel column. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol, 80%) was eluted with 10% MeOH in CH₂Cl₂. 2'-Deoxy-2'-methylene-4-N-acetyl-cytidine (0.56 g, 1.99 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.81 g, 2.4 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃ (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield **21** (0.88 g, 1.5 mmol, 75%).

Example 51: 1-(2'-Deoxy-2'-Methylene-5'-O-Dimethoxytrityl-β-D-ribofurano-
syl)-4-N-Acetyl-Cytosine 3'-(2-Cyanoethyl-N,N-diisopropylphosphoramidite)
(**22**)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-4-N-acetyl-cytosine **21** (0.88 g, 1.5 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.8 mL, 4.5 mmol) was added, followed by the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture

was stirred 2 h at room temperature and quenched with ethanol (1 mL). After 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). The product **22** (0.82 g, 1.04 mmol, 69%) was purified by flash chromatography over silica gel using 50-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.36 (CH₂Cl₂:MeOH / 20:1).

Example 52: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropyl disiloxane-1,3-diyl)-4-N-Acetyl-Cytidine (24)

Et₃N (6.9 mL, 50 mmol) was added to a solution of POCl₃ (0.94 mL, 10 mmol) and 1,2,4-triazole (3.1 g, 45 mmol) in acetonitrile (20 mL) at 0 °C. A solution of 2'-deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)uridine **23** ([described in example 45] 2.6 g, 5 mmol) in acetonitrile (20 mL) was added dropwise to the above reaction mixture and left to stir at RT for 4 h. The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄ concentrated *in vacuo*, dissolved in dioxane (20 mL) and aq. ammonia (30 mL). The mixture was stirred for 12 h and concentrated *in vacuo*. The residue was azeotroped with anhydrous pyridine (2 x 20 mL). Acetic anhydride (5 mL) was added to the residue dissolved in pyridine, stirred at RT for 4 h and quenched with sat. NaHCO₃ (5mL). The mixture was concentrated *in vacuo*, dissolved in CH₂Cl₂ (2 x 100 mL) and washed with 5% NaHCO₃ (1 x 100 mL). The organic extracts were dried over Na₂SO₄, concentrated *in vacuo* and the residue chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine **24** (2.2 g, 3.9 mmol, 78%) was eluted with 20% EtOAc in hexanes.

Example 53: 1-(2'-Deoxy-2'-Difluoromethylene-5'-O-Dimethoxytrityl-β-D-ribofuranosyl)-4-N-Acetyl-Cytosine (25)

2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-4-N-acetyl-cytidine **24** (2.2 g, 3.9 mmol) dissolved in THF (20 mL) was treated with 1 M TBAF in THF (3 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-4-N-acetyl-cytidine (0.89 g, 2.8 mmol, 72%) was eluted with 10% MeOH in CH₂Cl₂. 2'-Deoxy-2'-difluoromethylene-

4-*N*-acetyl-cytidine (0.89 g, 2.8 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (1.03 g, 3.1 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃ (50 mL), water (50 mL) and brine (50 mL). The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using EtOAc:hexanes / 60:40 as eluant to yield 25 (1.2 g, 1.9 mmol, 68%).

10 Example 54: 1-(2'-Deoxy-2'-difluoromethylene-5'-*O*-Dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-Acetylcytosine 3'-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) (26)

15 1-(2'-Deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-4-*N*-acetylcytosine 25 (0.6 g, 0.97 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (0.5 mL, 2.9 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.4 mL, 1.8 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product 26, a white foam (0.52 g, 0.63 mmol, 65%) was purified by flash chromatography over silica gel using 30-70% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.48 (CH₂Cl₂:MeOH / 20:1).

Example 55: 2'-Keto-3',5'-*O*-(Tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine (28)

25 Acetic anhydride (4.6 mL) was added to a solution of 3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine (Brown, J.; Christodolou, C.; Jones, S.; Modak, A.; Reese, C.; Sibanda, S.; Ubasawa A. *J. Chem. Soc. Perkin Trans. I* 1989, 1735) (6.2 g, 9.2 mmol) in DMSO (37 mL) and the resulting mixture was stirred at room temperature for 24 h. The mixture was concentrated *in vacuo*. The residue was taken up in EtOAc and washed with water. The organic layer was dried over MgSO₄ and concentrated *in vacuo*. The residue was purified on a silica gel column to yield 2'-keto-3',5'-*O*-(tetraisopropylidisiloxane-1,3-diyl)-6-*N*-(4-*t*-butylbenzoyl)-adenosine 28 (4.8 g, 7.2 mmol, 78%).

Example 56: 2'-Deoxy-2'-methylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)

Under a pressure of argon, sec-butyllithium in hexanes (11.2 mL, 14.6 mmol) was added to a suspension of triphenylmethylphosphonium iodide (7.07 g, 17.5 mmol) in THF (25 mL) cooled at -78 °C. The homogeneous orange solution was allowed to warm to -30 °C and a solution of 2'-keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 28 (4.87 g, 7.3 mmol) in THF (25 mL) was transferred to this mixture under argon pressure. After warming to RT, stirring was continued for 24 h. THF was evaporated and replaced by CH₂Cl₂ (250 mL), water was added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H₂O (20 mL), 5% aqueous NaHCO₃ (20 mL), H₂O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give the crude compound, which was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine 29 (3.86 g, 5.8 mmol, 79%).

Example 57: 2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine

2'-Deoxy-2'-methylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-*t*-butylbenzoyl)-adenosine (3.86 g, 5.8 mmol) dissolved in THF (30 mL) was treated with 1 M TBAF in THF (15 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (1.8 g, 4.3 mmol, 74%) was eluted with 10% MeOH in CH₂Cl₂.

Example 58: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-*t*-Butylbenzoyl)-Adenosine (29)

2'-Deoxy-2'-methylene-6-N-(4-*t*-butylbenzoyl)-adenosine (0.75 g, 1.77 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl (0.66 g, 1.98 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in

CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as an eluant to yield 29 (0.81 g, 1.1 mmol, 62%).

5 Example 59: 5'-O-DMT-2'-Deoxy-2'-Methylene-6-N-(4-t-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl N,N-diisopropylphosphoramidite) (31)

1-(2'-Deoxy-2'-methylene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-6-N-(4-t-butylbenzoyl)-adenine 29 dissolved in dry CH₂Cl₂ (15 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine was added, followed by
10 the dropwise addition of 2-cyanoethyl N,N-diisopropylchlorophosphoramidite. The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). The product was purified by flash chromatography over silica gel using 30-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant (0.7 g, 0.76
15 mmol, 68%). R_f 0.45 (CH₂Cl₂: MeOH / 20:1)

Example 60: 2'-Deoxy-2'-Difluoromethylene-3',5'-O-(Tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-t-Butylbenzoyl)-Adenosine

2'-Keto-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine 28 (6.7 g, 10 mmol) and triphenylphosphine (2.9 g, 11 mmol) were
20 dissolved in diglyme (20 mL), and heated to a bath temperature of 160 °C. A warm (60 °C) solution of sodium chlorodifluoroacetate (2.3 g, 15 mmol) in diglyme (50 mL) was added (dropwise from an equilibrating dropping funnel) over a period of ~1 h. The resulting mixture was further stirred for 2 h and concentrated *in vacuo*. The residue was dissolved in CH₂Cl₂ and
25 chromatographed over silica gel. 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine (4.1g, 6.4 mmol, 64%) eluted with 15% hexanes in EtOAc.

Example 61: 2'-Deoxy-2'-Difluoromethylene-6-N-(4-t-Butylbenzoyl)-Adenosine

30 2'-Deoxy-2'-difluoromethylene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-6-N-(4-t-butylbenzoyl)-adenosine (4.1 g, 6.4 mmol) dissolved in THF (20 mL)

was treated with 1 M TBAF in THF (10 mL) for 20 m and concentrated *in vacuo*. The residue was triturated with petroleum ether and chromatographed on a silica gel column. 2'-Deoxy-2'-difluoromethylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol, 77%) was eluted with 20% MeOH in CH₂Cl₂.

5 Example 62: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine (30)

2'-Deoxy-2'-difluoromethylene-6-*N*-(4-*t*-butylbenzoyl)-adenosine (2.3 g, 4.9 mmol) was dissolved in pyridine (10 mL) and a solution of DMT-Cl in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was
10 stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 50% EtOAc:hexanes as eluant to yield 30 (2.6 g, 3.41
15 mmol, 69%).

Example 63: 5'-O-DMT-2'-Deoxy-2'-Difluoromethylene-6-*N*-(4-*t*-Butylbenzoyl)-Adenosine 3'-(2-Cyanoethyl *N,N*-diisopropylphosphoramidite) (32)

1-(2'-Deoxy-2'-difluoromethylene-5'-*O*-dimethoxytrityl-β-D-ribofuranosyl)-6-*N*-(4-*t*-butylbenzoyl)-adenine 30 (2.6 g, 3.4 mmol) dissolved in dry CH₂Cl₂
20 (25 mL) was placed in a round bottom flask under Ar. Diisopropylethylamine (1.2 mL, 6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (1.06 mL, 4.76 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After
25 10 m the mixture evaporated to a syrup *in vacuo* (40 °C). 32 (2.3 g, 2.4 mmol, 70%) was purified by flash column chromatography over silica gel using 20-50% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. R_f 0.52 (CH₂Cl₂: MeOH / 15:1).

Example 64: 2'-Deoxy-2'-Methoxycarbonylmethylidene-3',5'-*O*-(Tetraiso-propyldisiloxane-1,3-diyl)-Uridine (33)

30 Methyl(triphenylphosphoranylidene)acetate (5.4 g, 16 mmol) was added to a solution of 2'-keto-3',5'-*O*-(tetraisopropyl disiloxane-1,3-diyl)-uridine 14 in

- CH₂Cl₂ under argon. The mixture was left to stir at RT for 30 h. CH₂Cl₂ (100 mL) and water were added (20 mL), and the solution was neutralized with a cooled solution of 2% HCl. The organic layer was washed with H₂O (20 mL), 5% aq. NaHCO₃ (20 mL), H₂O to neutrality, and brine (10 mL). After drying (Na₂SO₄), the solvent was evaporated *in vacuo* to give crude product, that was chromatographed on a silica gel column. Elution with light petroleum ether:EtOAc / 7:3 afforded pure 2'-deoxy-2'-methoxycarbonylmethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5.8 g, 10.8 mmol, 67.5%).

Example 65: 2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (34)

- Et₃N•3 HF (3 mL) was added to a solution of 2'-deoxy-2'-methoxycarboxymethylidene-3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-uridine **33** (5 g, 9.3 mmol) dissolved in CH₂Cl₂ (20 mL) and Et₃N (15 mL). The resulting mixture was evaporated *in vacuo* after 1 h and chromatographed on a silica gel column eluting 2'-deoxy-2'-methoxycarbonylmethylidene-uridine **34** (2.4 g, 8 mmol, 86%) with THF:CH₂Cl₂ / 4:1.

Example 66: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine (35)

- 2'-Deoxy-2'-methoxycarbonylmethylidene-uridine **34** (1.2 g, 4.02 mmol) was dissolved in pyridine (20 mL). A solution of DMT-Cl (1.5 g, 4.42 mmol) in pyridine (10 mL) was added dropwise over 15 m. The resulting mixture was stirred at RT for 12 h and MeOH (2 mL) was added to quench the reaction. The mixture was concentrated *in vacuo* and the residue taken up in CH₂Cl₂ (100 mL) and washed with sat. NaHCO₃, water and brine. The organic extracts were dried over MgSO₄, concentrated *in vacuo* and purified over a silica gel column using 2-5% MeOH in CH₂Cl₂ as an eluant to yield 5'-O-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine **35** (2.03 g, 3.46 mmol, 86%).

Example 67: 5'-O-DMT-2'-Deoxy-2'-Methoxycarbonylmethylidene-Uridine 3'-(2-cyanoethyl-N,N-diisopropylphosphoramidite) (36)

- 1-(2'-Deoxy-2'-2'-methoxycarbonylmethylidene-5'-O-dimethoxytrityl-β-D-ribofuranosyl)-uridine **35** (2.0 g, 3.4 mmol) dissolved in dry CH₂Cl₂ (10 mL) was placed in a round-bottom flask under Ar. Diisopropylethylamine (1.2 mL,

6.8 mmol) was added, followed by the dropwise addition of 2-cyanoethyl *N,N*-diisopropylchlorophosphoramidite (0.91 mL, 4.08 mmol). The reaction mixture was stirred 2 h at RT and quenched with ethanol (1 mL). After 10 m the mixture was evaporated to a syrup *in vacuo* (40 °C). 5'-*O*-DMT-2'-deoxy-2'-methoxycarbonylmethylidene-uridine 3'-(2-cyanoethyl-*N,N*-diisopropylphosphoramidite) **36** (1.8 g, 2.3 mmol, 67%) was purified by flash column chromatography over silica gel using a 30-60% EtOAc gradient in hexanes, containing 1% triethylamine, as eluant. *R*_f 0.44 (CH₂Cl₂:MeOH / 9.5:0.5).

10 Example 68: 2'-Deoxy-2'-Carboxymethylidene-3',5'-*O*-(Tetraisopropyl-disiloxane-1,3-diyl)-Uridine **37**

2'-Deoxy-2'-methoxycarbonylmethylidene-3',5'-*O*-(tetraisopropyl-disiloxane-1,3-diyl)-uridine **33** (5.0 g, 10.8 mmol) was dissolved in MeOH (50 mL) and 1 N NaOH solution (50 mL) was added to the stirred solution at RT. The mixture was stirred for 2 h and MeOH removed *in vacuo*. The pH of the aqueous layer was adjusted to 4.5 with 1N HCl solution, extracted with EtOAc (2 x 100 mL), washed with brine, dried over MgSO₄ and concentrated *in vacuo* to yield the crude acid. 2'-Deoxy-2'-carboxymethylidene-3',5'-*O*-(tetraisopropyl-disiloxane-1,3-diyl)-uridine **37** (4.2 g, 7.8 mmol, 73%) was purified on a silica gel column using a gradient of 10-15% MeOH in CH₂Cl₂.

20 Example 69: Synthesis of 2'-*C*-allyl-U phosphoramidite from 5'-*O*-DMT-3'-*O*-TBDMS-Uridine.

Referring to Figure 54, in order to simplify the synthetic scheme for phosphoramidites **5** and **8** we also explored the potential of 5'-*O*-DMT-3'-*O*-TBDMS-Uridine **10** (side product in preparation of standard RNA monomers) as a starting material in the synthesis of key intermediate **4**. Phenoxythiocarbonylation of starting synthon **10** according to Robins (Robins, M. J., Wilson J. S. and Hansske, F. (1983), *J. Am. Chem. Soc.*, 105, 4059) surprisingly led to thioester **11** (91 %) without noticeable migration (Scaringe, S.A., Franclyn, C. & Usman, N. (1990) *Nucleic Acids Res.*, 18, 5433-5441) of the TBDMS group. Comparative analysis of ¹H NMR data for compounds **10** and **11** revealed that resonance of H-2' experienced up field shift of 2,0 ppm(from 6,06 to 4,13) in **11** compare to starting compound **10**, at the same time chemical shift of H-3' and H-1' changed only slightly: 4.83 ppm(H-3') and

6.48 ppm (H-1') in 11 compare to 4.36(H-3') ppm and 5.93 ppm (H-1') in 10 and chemical shift of H-4' remains practically unchanged indicating acylation at C-2-OH. Heck allylation of intermediate 11 with 2-,2'-Azobis-(2-methyl propionitrile) (other groups can be introduced by standard procedures) resulted in a formation of 2'-C-allyl derivative 12 (70 %) and related 2'-deoxy by-product (15%). Subsequent desilylation of 12 led to 5'-O-DMT derivative 4 identical to the one synthesized from thioester 2. Since the starting material for this route is commercially available this may represent a less laborious way to key synthon 4 as well as for other 2'- modified monomers. This methodology can be used to introduce other 2'-C-allyl groups using compound 11 (or its equivalent for other bases) as an intermediate.

Example 70: Synthesis of 5'-O-Dimethoxytrityl-2'-O-Phenoxythiocarbonyl-3'-O-t-butylidimethylsilyl-uridine 11.

To a stirred solution of 5'-O-Dimethoxytrityl-3'-O-t-butylidimethylsilyl-uridine (Commercially available from Chem Genes Corporation) (5,0 g 7,57 mmol) and dimethylaminopyridine (1,8g, 15 mmol) in 100 ml of dry acetonitril a solution of phenylchlorothionoformate (1.26ml, 9,1 mmol) in 25 ml of acetonitrile was added dropwise and the reaction mixture stirred at room temperature for 3 hours. TLC (ethylacetate-hexanes 1:1) showed disappearance of starting material and the reaction mixture was concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel CH₂Cl₂ as an eluent to give 5.51g (91.3%) of the product.

¹H NMR (CDCl₃) δ 0.95 (s, 9H, tBu), 0.11 (s, 3H, CH₃), 0.04 (s, 3H, CH₃) 3.57 (2H, H5', H5'', m J_{5',4'}=2.4., J_{5'',4'}=2,8., J_{5',5''}=11.0), 3.86 (6H, OCH₃, s), 4.07 (1H, H4', m), 4.83 (1H, H3', dd, J_{3',4'}=2,8 J_{3',2'}=5,2), 5.44 (1H, H5, d, J_{5,6}=8.0) 5.99 (1H, H2', dd, J_{2',1'}=6.4 , J_{2',3'}= 5,2), 6.46 (1H, H1', d, J_{1',2'}=6.4) , 6.89-7.79 (18H, DMT, Phe, m), 7.88 (1H, H6, d, J_{6,5}=8.0), 7.95 (1H, N-H, bs).

Example 71: Synthesis of 5'-O-Dimethoxytrityl-2'-C-Allyl-3'-O-t-butylidimethylsilyl-uridine(12)

To a refluxing under argon solution of 5'-O-Dimethoxytrityl-2'-O-Phenoxythiocarbonyl-3'-O-t-butylidimethylsilyl-uridine (5,5g, 6,9 mmol) and

- allyltributyltin (10,7ml, 34,5 mmol) in dry toluene (150 ml) a solution of 2,2'-Azobis-(2-methyl propionitrile) (0.28g 1,72 mmol) in 50 ml of dry toluene was added dropwise for 1 hour. The resulting mixture was allowed to reflux under argon for additional 2 hours. After that it was concentrated in vacuo and
- 5 purified by flash chromatography on silica gel with gradient ethylacetate in hexanes (0-30%) as an eluent. Yield 3.38g (70.0%).

¹H NMR (CDCl₃) δ 0.95 (s, 9H, tBu), 0.11 (s, 3H, CH₃), 0.04 (s, 3H, CH₃), 2.23 (1H, H6', m), . 2.38-2.52 (2H, H6" and H2', m), 3.46 (2H, H5' and H5", m, J_{5',4'}=2.5., J_{5'',4'}=3.2 J_{5',5''}=10.8), 3.86 (6H, OCH₃, s), 4.13 (1H, H4', dd, J_{4',3'}=8.0, J_{4',5'}=3.2, J_{4',5''}=2.5), 4.46 (1H, H3', m), 5.15 (1H, H8', d, J_{8',7'}=10.0), 5.20 (1H, H9', d, J_{9',7'}=17.3), 5.44 (1H, H5, d, J_{5,6}=8.0), 5.81 (1H, H7', dddd, J_{7',6'}=6.0, J_{7',6''}=8.0), 6.14 (1H, H1', d, J_{1',2'}=8.0), 6.88-7.52 (13H, DMT, m), 7.76 (1H, H6, d, J_{6,5}=8.0), 8.17 (1H, N-H, bs)

10

- Example 72: Synthesis of 5'-O-Dimethoxytrityl-2'-C-Allyl Uridine (4) from 5'-O-Dimethoxytrityl-2'-C-Allyl-3'-O-t-butylidimethyl-silyl-uridine (12).
- 15

Standard deprotection of TBDMS derivative 12 utilizing general method A furnished product 4 (yield 80%) identical to the compound prepared from 2'-C-allyl derivative 3.

Uses

- 20 The alkyl substituted nucleotides of this invention can be used to form stable oligonucleotides as discussed above for use in enzymatic cleavage or antisense situations. Such oligonucleotides can be formed enzymatically using triphosphate forms by standard procedure. Administration of such oligonucleotides is by standard procedure. See Sullivan *et al.* PCT WO
- 25 94/02595.

The following are non-limiting examples showing the synthesis of nucleic acids using 2'-O-methylthioalkyl-substituted phosphoramidites and the syntheses of the amidites.

Example 73: Synthesis of Hammerhead Ribozymes Containing 2'-O-alkylthioalkylnucleotides & Other Modified Nucleotides

The method of synthesis follows the procedure for normal RNA synthesis as described in Usman, N.; Ogilvie, K.K.; Jiang, M.-Y.; Cedergren, R.J. *J. Am. Chem. Soc.* **1987**, *109*, 7845-7854 and in Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* **1990**, *18*, 5433-5441 and makes use of common nucleic acid protecting and coupling groups, such as dimethoxytrityl at the 5'-end, and phosphoramidites at the 3'-end. These 2'-O-alkylthioalkyl substituted phosphoramidites may be incorporated not only into hammerhead ribozymes, but also into hairpin, hepatitis delta virus, Group I or Group II intron catalytic nucleic acids, or into antisense oligonucleotides. They are, therefore, of general use in any nucleic acid structure.

Example 74: Synthesis of base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) nucleosides (2)

Referring to Figure 55, standard introduction of "Markiewicz" protecting group to the base-protected nucleosides according to "Oligonucleotides and Analogues. A Practical Approach", ed. F. Eckstein, IRL Press, 1991 resulted in protected nucleosides (2) with 85-100% yields. Briefly, in a non-limiting example, Uridine (20g, 81.9 mmol) was dried by two coevaporations with anhydrous pyridine and re dissolved in the anhydrous pyridine. The above solution was cooled (0°C) and solution of 1,3-dichloro-1,1,3,3-tetraisopropylsiloxane (28.82 mL, 90.09 mmol) in 30 mL of anhydrous dichloroethane was added dropwise under stirring. After the addition was completed the reaction mixture was allowed to warm to room temperature and stirred for additional two hours. Then it was quenched with MeOH (25 mL) and evaporated to dryness. The residue was dissolved in methylene chloride and washed with saturated NaHCO₃ and brine. The organic layer was evaporated to dryness and then coevaporated with toluene to remove traces of pyridine to give 39g (98%) of compound 2 (B=Ura) which was used without further purification.

Other 3',5'-O-(tetraisopropylidisiloxane-1,3-di-yl)- nucleosides were obtained in 75-90% yields, using the protocol described above, starting from

base-protected nucleosides with final purification of the products by flash chromatography on silica gel when necessary.

Example 75: General procedure for the synthesis of 2'-O-methylthiomethyl nucleosides (3)

- 5 Referring to Figure 55, to a stirred ice-cooled solution of the mixture of base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) nucleoside (2) (7 mmol), methyl disulfide (70 mmol), 2,6-lutidine (7 mmol) in methylene chloride (100 mL) or mixture methylene chloride - acetonitrile (1:1) under positive pressure of argon, solution of benzoyl peroxide (28 mmol) in methylene chloride was added dropwise during 1 hour. After complete addition the reaction mixture was stirred at 0°C under argon for additional 1 hour. The solution was allowed to warm to room temperature, diluted with methylene chloride (100 mL), washed twice with saturated aq NaHCO₃ and brine. The organic layer was dried over sodium sulfate and evaporated to dryness.
- 10 The residue was purified by flash chromatography on silica using 1-2% methanol in methylene chloride as an eluent to give corresponding methylthiomethyl nucleosides with 55-70% yield.
- 15

Example 76: 5'-O-Dimethoxytrityl-2'-O-Methylthiomethyl-Nucleosides. (6)

- 20 **Method A.** The solution of the base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl)-2'-O-methylthiomethyl nucleoside (3) (2.00 mmol) in 10 ml of dry tetrahydrofuran (THF) was treated with 1M solution of tetrabutylammoniumfluoride in THF (3.0 ml) for 10-15 minutes at room temperature. Resulting mixture was evaporated, the residue was loaded to the silica gel column, washed with 1L of chloroform, and the desired deprotected compound was eluted with 5-10% methanol in dichloromethane. Appropriate fractions were combined, solvents removed by evaporation, and the residue was dried by coevaporation with dry pyridine. The oily residue was redissolved in dry pyridine, dimethoxytritylchloride (1.2 eq) was added and the reaction mixture was left under anhydrous conditions overnight. The reaction was quenched with methanol (20 ml), evaporated, dissolved in chloroform, washed with saturated aq sodium bicarbonate and brine. Organic layer was dried over sodium sulfate and evaporated. The residue was purified
- 25
- 30

by flash chromatography on silica gel to give 5'-O-Dimethoxytrityl derivatives with 70-80% yield.

Method B. Alternatively, 5'-O-Dimethoxytrityl-2'-O-Methylthiomethyl-Nucleosides (6) may also be synthesized using 5'-O-Dimethoxytrityl-3'-O-t-Butyl-dimethylsilyl Nucleosides (4) as the starting material. Compound 4 is commercially available as a by-product during RNA phosphoramidite synthesis. Compound 4 is converted in to 3'-O-t-butyl dimethylsilyl-2'-O-methylthiomethyl nucleoside 5, as described under example 3. The solution of the base-protected 3'-O-t-butyl dimethylsilyl-2'-O-methylthiomethyl nucleoside 5 (2.00 mmol) in 10 ml of dry tetrahydrofuran (THF) was treated with 1M solution of tetrabutylammoniumfluoride in THF (3.0 ml) for 10-15 minutes at room temperature. The resulting mixture was evaporated, and purified by flash silica gel chromatography to give nucleosides 6 in 90% yield.

Example 77: 5'-O-Dimethoxytrityl-2'-O-Methylthiomethyl-Nucleosides-3'-(2-Cyanoethyl-N,N-diisopropylphosphoramidites) (7)

Standard phosphitylation of nucleoside 6 according to Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 yielded phosphoramidites in 70-85% yield.

Example 78: General procedure for the synthesis of 2'-O-Methylthiophenyl nucleosides.

To a stirred ice-cooled solution of the mixture of base-protected 3',5'-O-(tetraisopropylidisiloxane-1,3-diyl) nucleoside (14.7 mmol), thioanisole (147 mmol), N,N-dimethylaminopyridine (58.8 mmol) in acetonitrile (100 mL) under positive pressure of argon, benzoyl peroxide (36.75 mmol) was added portionwise over 3 hours. After complete addition the reaction mixture was allowed to warm to room temperature and was stirred under argon for an additional 1 hour. The solvents were removed in vacuo, the residue was dissolved in ethylacetate, washed twice with saturated aq NaHCO₃ and brine. The organic layer was dried over sodium sulfate and evaporated to dryness. The residue was purified by flash chromatography on silica using mixture EtOAc-hexanes (1:1) as eluent to give the corresponding methylthiophenyl nucleosides with 55-65% yield.

Example 79: 5'-O-Dimethoxytrityl-2'-O-Methylthiophenyl-Nucleosides.

These compounds were prepared as described above under examples 76 and 76.

Example 80: 5'-O-Dimethoxytrityl-2'-O-Methylthiophenyl-Nucleosides-3'-(2-Cyanoethyl N,N-diisopropylphosphoramidites)

Standard phosphitylation according to Scaringe, S.A.; Franklyn, C.; Usman, N. *Nucleic Acids Res.* 1990, 18, 5433-5441 yielded phosphoramidites in 70-85% yield.

Example 81: Ribozymes containing 2'-O-methylthiomethyl substitutions

10 In a non-limiting example 2'-O-methylthioalkyl substitutions were made at various positions within a hammerhead ribozyme motif (Fig. 56, including U4 and U7 positions). The target site B was targeted by the hammerhead ribozyme in this non-limiting example.

15 Hammerhead ribozymes (see Fig. 56) were synthesized using solid-phase synthesis, as described above. Several positions were modified, individually or in combination, with 2'-O-methylthiomethyl groups.

RNA cleavage assay *in vitro*:

20 Substrate RNA is 5' end-labeled using [γ -³²P] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme are denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10-15 min. The ribozyme and substrate are incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl₂. The reaction is
25 initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μ l are taken at regular intervals of time and the reaction is quenched by mixing with equal volume of 2X formamide stop mix. The samples are resolved on 20 % denaturing polyacrylamide gels. The results are quantified and percentage of target RNA cleaved is plotted as a function of
30 time.

Referring to Figure 57, hammerhead ribozymes containing 2'-O-methylthiomethyl modifications at various positions cleave the target RNA efficiently. Surprisingly, all the 2'-O-methylthiomethyl -substituted ribozymes cleaved the target RNA more efficiently compared to the control hammerhead ribozyme.

Sequences listed in Figure 56 and the modifications described in Figure 56 and 57 are meant to be non-limiting examples. Those skilled in the art will recognize that variants (base-substitutions, deletions, insertions, mutations, chemical modifications) of the ribozyme and RNA containing other combinations of 2'-hydroxyl group modifications can be readily generated using techniques known in the art, and are within the scope of the present invention.

The following are non-limiting examples showing the synthesis of non-nucleotide mimetic-containing catalytic nucleic acids using non-nucleotide phosphoramidites.

Such non-nucleotides can be located in the binding arms, core or the loop adjacent stem II of a hammerhead type ribozyme. Those in the art following the teachings herein can determine optimal locations in these regions. Surprisingly, abasic moieties can be located in the core of such a ribozyme.

Example 82: Synthesis of Abasic nucleotides

The synthesis of 1-deoxy-D-ribofuranose phosphoramidite **9** is shown in Figure 58. Our initial efforts concentrated on the deoxygenation of synthon **1**, prepared by a "one pot" procedure from D-ribose. Phenoxythiocarbonylation of acetonide **1** under Robins conditions led to the β -anomer **2** ($J_{1,2} = 1.2$ Hz) in modest yield (45-55%). Radical deoxygenation using $\text{Bu}_3\text{SnH/AIBN}$ resulted in the formation of the ribitol derivative **3** in 50% yield. Subsequent deprotection with 90% CF_3COOH (10 m) and introduction of a dimethoxytrityl group led to the key intermediate **4** in 40% yield (Yang et al., *Biochemistry* 1992, 31, 5005-5009; Perreault et al., *Biochemistry* 1991, 30, 4020-4025; Paoletta et al., *EMBO J.* 1992, 11, 1913-1919; Peiken et al., *Science* 1991, 253, 314-317).

The low overall yield of this route prompted us to investigate a different approach to 4 (Fig. 58). Phenylthioglycosides, successfully employed in the Keck reaction, appeared to be an alternative. However, it is known that free-radical reduction of the corresponding glycosyl bromides with participating acyl groups at the C2-position can result in the migration of the 2-acyl group to the C1-position (depending on Bu_3SnH concentration). Therefore we subjected phenylthioglycoside 5 to radical reduction with Bu_3SnH (6.1 eq.) in the presence of Bz_2O_2 (2 eq.) resulting in the isolation of tribenzoate 6 in 63% yield (Fig. 9B). Subsequent debenzoylation and dimethoxytritylation led to synthon 4 in 70% yield. Introduction of the TBDMS group, using standard conditions, resulted in the formation of a 4:1 ratio of 2- and 3-isomers 8 and 7. The two regioisomers were separated by silica gel chromatography. The 2-O-t-butyltrimethylsilyl derivative 8 was phosphitylated to provide phosphoramidite 9 in 82% yield.

15 Example 83: RNA cleavage assay *in vitro*

Ribozymes and substrate RNAs were synthesized as described above. Substrate RNA was 5' end-labeled using [$\gamma\text{-}^{32}\text{P}$] ATP and T4 polynucleotide kinase (US Biochemicals). Cleavage reactions were carried out under ribozyme "excess" conditions. Trace amount (≤ 1 nM) of 5' end-labeled substrate and 40 nM unlabeled ribozyme were denatured and renatured separately by heating to 90°C for 2 min and snap-cooling on ice for 10 -15 min. The ribozyme and substrate were incubated, separately, at 37°C for 10 min in a buffer containing 50 mM Tris-HCl and 10 mM MgCl_2 . The reaction was initiated by mixing the ribozyme and substrate solutions and incubating at 37°C. Aliquots of 5 μl are taken at regular intervals of time and the reaction quenched by mixing with an equal volume of 2X formamide stop mix. The samples were resolved on 20 % denaturing polyacrylamide gels. The results were quantified and percentage of target RNA cleaved is plotted as a function of time.

30 Referring to Figure 59 there is shown the general structure of a hammerhead ribozyme targeted against site B (HH-B) with various bases numbered. Various substitutions were made at several of the nucleotide positions in HH-B. Specifically referring to Figure 60, substitutions were made

at the U4 and U7 positions marked as X4 and X7 and also in loop II in the positions marked by an X. The RNA cleavage activity of these substituted ribozymes is shown in the following figures. Specifically, Figure 61 shows cleavage by an abasic substituted U4 and an abasic substituted U7. As will be noted, abasic substitution at U4 or U7 does not significantly affect cleavage activity. In addition, inclusion of all abasic moieties in stem II loop does not significantly reduce enzymatic activity as shown in Figure 62. Further, inclusion of a 3' inverted deoxyribose does not inactivate the RNA cleavage activity as shown in Figure 63.

10 Example 84: Smooth Muscle Cell Proliferation Assay

Hammerhead ribozyme (HH-A) is targeted to a unique site (site A) within *c-myb* mRNA. Expression of *c-myb* protein has been shown to be essential for the proliferation of rat smooth muscle cell (Brown et al., 1992 *J. Biol. Chem.* 267, 4625).

15 The ribozymes that cleaved site A within *c-myb* RNA described above were assayed for their effect on smooth muscle cell proliferation. Rat vascular smooth muscle cells were isolated and cultured as described (Stnchcomb et al., *supra*). These primary rat aortic smooth muscle cells (RASMC) were plated in a 24-well plate (5×10^3 cells/well) and incubated at 37°C in the presence of Dulbecco's Minimal Essential Media (DMEM) and 10% serum for ~16 hours.

25 These cells were serum-starved for 48-72 hours in DMEM (containing 0.5% serum) at 37°C. Following serum-starvation, the cells were treated with lipofectamine (LFA)-complexed ribozymes (100 nM ribozyme was complexed with LFA such that LFA:ribozyme charge ration is 4:1).

30 Ribozyme:LFA complex was incubated with serum-starved RASMC cells for four hours at 37°C. Following the removal of ribozyme:LFA complex from cells (after 4 hours), 10% serum was added to stimulate smooth cell proliferation. Bromo-deoxyuridine (BrdU) was added to stain the cells. The cells were stimulated with serum for 24 hours at 37°C.

Following serum-stimulation, RASMC cells were quenched with hydrogen peroxide (0.3% H₂O₂ in methanol) for 30 min at 4°C. The cells were then denatured with 0.5 ml 2N HCl for 20 min at room temperature. Horse serum (0.5 ml) was used to block the cells at 4°C for 30 min up to ~16
5 hours.

The RASMC cells were stained first by treating the cells with anti-BrdU (primary) antibody at room temperature for 60 min. The cells were washed with phosphate-buffered saline (PBS) and stained with biotinylated affinity-purified anti-mouse IgM (Pierce, USA) secondary antibody. The cells were
10 counterstained using avidin-biotinylated enzyme complex (ABC) kit (Pierce, USA).

The ratio of proliferating:non-proliferating cells was determined by counting stained cells under a microscope. Proliferating RASMCs will incorporate BrdU and will stain brown. Non-proliferating cells do not
15 incorporate BrdU and will stain purple.

Referring to Figure 64 there is shown a ribozyme which cleaves the site A referred to as HH-A. Substitutions of abasic moieties in place of U4 as shown in Figure 65 provided active ribozyme as shown in Figure 66 using the above-noted rat aortic smooth muscle cell proliferation assay.

20 The method of this invention generally features HPLC purification of ribozymes. An example of such purification is provided below in which a synthetic ribozyme produced on a solid phase is blocked. This material is then released from the solid phase by a treatment with methanolic ammonia, subsequently treated with tetrabutylammonium fluoride, and purified on
25 reverse phase HPLC to remove partially blocked ribozyme from "failure" sequences. Such "failure" sequences are RNA molecules which have a nucleotide base sequence shorter to that of the desired enzymatic RNA molecule by one or more of the desired bases in a random manner, and possess free terminal 5'-hydroxyl group. This terminal 5'-hydroxyl in a
30 ribozyme with the correct sequence is still blocked by lipophilic dimethoxytrityl group. After such partially blocked enzymatic RNA is purified, it is deblocked by a standard procedure, and passed over the same or a similar HPLC

reverse phase column to remove other contaminating components, such as other RNA molecules or nucleotides or other molecules produced in the deblocking and synthetic procedures. The resulting molecule is the native enzymatically active ribozyme in a highly purified form.

- 5 Below are provided examples of such a method. These examples can be readily scaled up to allow production and purification of gram or even kilogram quantities of ribozymes.

Example 85: HPLC Purification, Reverse-Phase

- 10 In this example solid phase phosphoramidite chemistry was employed for synthesis of a ribozyme. Monomers used were 2'-*t*-butyl-dimethylsilyl cyanoethylphosphoramidites of uridine, *N*-benzoyl-cytosine, *N*-phenoxyacetyl adenosine, and guanosine (Glen Research, Sterling, VA).

- 15 Solid phase synthesis was carried out on either an ABI 394 or 380B DNA/RNA synthesizer using the standard protocol provided with each machine. The only exception was that the coupling step was increased from 10 to 12 minutes. The phosphoramidite concentration was 0.1 M. Synthesis was done on a 1 μ mol scale using a 1 μ mol RNA reaction column (Glen Research). The average coupling efficiencies were between 97% and 98% for the 394 model and between 97% and 99% for the 380B model, as
20 determined by a calorimetric measurement of the released trityl cation. The final 5'-DMT group was not removed.

- 25 After synthesis, the ribozymes were cleaved from the CPG support, and the base and phosphotriester moieties were deprotected in a sterile vial by incubation in dry ethanolic ammonia (2 mL) at 55 °C for 16 hours. The reaction mixture was cooled on dry ice. Later, the cold liquid was transferred into a sterile screw cap vial and lyophilized.

- 30 To remove the 2'-*t*-butyldimethylsilyl groups from the ribozyme the obtained residue was suspended in 1 M tetra-*n*-butylammonium fluoride in dry THF (TBAF), using a 20-fold excess of the reagent for every silyl group, for 16 hours at ambient temperature. The reaction was quenched by adding an

equal volume of a sterile 1 M triethylamine acetate, pH 6.5. The sample was cooled and concentrated on a SpeedVac to half of the initial volume.

The ribozymes were purified in two steps by HPLC on a C4 300 Å 5 µm DeltaPak column in an acetonitrile gradient.

- 5 The first step, or "trityl on" step, was a separation of 5'-DMT-protected ribozyme(s) from failure sequences lacking a 5'-DMT group. Solvents used for this step were: A (0.1 M triethylammonium acetate, pH 6.8) and B (acetonitrile). The elution profile was: 20% B for 10 minutes, followed by a linear gradient of 20% B to 50% B over 50 minutes, 50% B for 10 minutes, a
10 linear gradient of 50% B to 100% B over 10 minutes, and a linear gradient of 100% B to 0% B over 10 minutes.

- The second step was a purification of a completely deprotected, *i.e.* following the removal of the 5'-DMT group, ribozyme by a treatment with 2% trifluoroacetic acid or 80% acetic acid on a C4 300 Å 5 µm DeltaPak column in
15 an acetonitrile gradient. Solvents used for this second step were: A (0.1 M Triethylammonium acetate, pH 6.8) and B (80% acetonitrile, 0.1 M triethylammonium acetate, pH 6.8). The elution profile was: 5% B for 5 minutes, a linear gradient of 5% B to 15% B over 60 minutes, 15% B for 10 minutes, and a linear gradient of 15% B to 0% B over 10 minutes.

- 20 The fraction containing ribozyme, which is in the triethylammonium salt form, was cooled and lyophilized on a SpeedVac. Solid residue was dissolved in a minimal amount of ethanol and ribozyme in sodium salt form was precipitated by addition of sodium perchlorate in acetone. (K^+ or Mg^{2+} salts can be produced in an equivalent manner.) The ribozyme was collected
25 by centrifugation, washed three times with acetone, and lyophilized.

Example 86: RNA and Ribozyme Deprotection of Exocyclic Amino Protecting Groups Using ethylamine (EA)

- The polymer-bound oligonucleotide, either trityl-on or off, was suspended in a solution of ethylamine (EA) @ 25-55 °C for 10-30 min to remove the
30 exocyclic amino protecting groups (see Figure 67). The supernatant was removed from the polymer support. The support was washed with 1.0 mL of

EtOH:MeCN:H₂O/3:1:1, vortexed and the supernatant was then added to the first supernatant. The combined supernatants, containing the oligoribonucleotide, were dried to a white powder.

5 Table EVII is a summary of the results obtained using the improvements outlined in this application for base deprotection. From this data it is evident EA at 55° for 10 m or 40° for 10 m is efficient. The HPLC peak structure is almost identical between these schemes, and the yield for the ethylamine deprotected oligos is actually slightly better than the methylamine.

10 The second step of the deprotection of RNA molecules may be accomplished by removal of the 2'-hydroxyl alkylsilyl protecting group using TBAF for 8-24 h (Usman *et al. J. Am. Chem. Soc.* 1987, 109, 7845-7854). Applicant has determined that the use of anhydrous TEA•HF in *N*-methylpyrrolidine (NMP) for 0.5-1.5 h @ 55-65 °C gives equivalent or better results.

15 The following are examples of preferred embodiments of the present invention. Those in the art will recognize that these are not limiting examples but rather are provided to guide those in the art to the full breadth of meaning of the present invention. Routine procedures can be used to utilize other coupling regions not exemplified below.

20 Ribozymes were synthesized in two parts and tested without ligation for catalytic activity. Referring to Fig. 72, the cleavage activity of the half ribozymes containing between 5 and 8 base pairs stem IIs at 40 nM under single turnover conditions was comparable to that of the full length oligomer as shown in Figs. 73 and 74. The same half ribozymes were synthesized with
25 suitable modifications at the nascent stem II loop to allow for crosslinking. The halves were purified and chemically ligated, using a variety of crosslinking methods. The resulting full length ribozymes (see Fig. 71) exhibited similar cleavage activity as the linearly synthesized full length oligomer as shown in Fig. 74.

Example 87

Referring to Fig. 70 the 5' half of a hammerhead ribozyme was provided with a ribose group. This was oxidatively cleaved with NaIO_4 and reacted with

- 5 The resulting ribozyme consisted of the two half ribozyme linked by a morpholino group.

- One equivalent of (200 micrograms) of 5' half hammerhead with a 3'OH and 5 equivalents (1000 micrograms) of 3' half with 5' C5-NH₂ all with HH-A were used in this reaction. The limiting oligonucleotide was oxidized first with
- 10 3.6 equivalents of sodium periodate for sixty minutes on ice in DEPC water quenched with 7.2 equivalents of ethylene glycol for 30 minutes on ice and the 5 equivalents of the amino oligo added. 0.5 Molar tricine buffer, pH 9, was added to provide 25 millimolar final tricine concentration and left for 30 minutes on ice. 50 equivalents of sodium cyanoborohydride was then added
- 15 and the pH reduced to 6.5 with acetic acid and reaction left for 60 minutes on ice. The resulting full length ribozyme was then purified for further analysis.

Example 88: Amide Bond

- Referring again to Fig. 70 and 71, a 5' half of ribozyme was provided with a carboxyl group at its 2' position and was coupled with an amine containing
- 20 3' half ribozyme. The provision of a coupling reagent resulted in a full-length ribozyme having an amide bond.

Example 89: Disulfide Bond

- Referring to Fig. 70 and 71, 250 micrograms of RPI3881 and 250 micrograms of RPI3636 half ribozyme were separately deprotected with
- 25 dithiothreitol overnight at 37°C. They were mixed together at 1:1 mole ratio in a 100 mM sodium phosphate buffer at pH 8 and 4M copper sulfate and 0.8 mM 1,10-phenanthroline (final concentrations) was added for two hours at room temperature (20-25°C) and the resulting mixture gel purified. The overall purification yield of full length ribozyme was 30%.

- 30 To make internally-labeled substrate RNA for trans-ribozyme cleavage reactions, a 1.8 KB region (containing site A) was synthesized by PCR using

primers that place the T7 RNA promoter upstream of the amplified sequence. Target RNA was transcribed, using T7 RNA polymerase, in a standard transcription buffer in the presence of [α - 32 P]CTP. The reaction mixture was treated with 15 units of ribonuclease-free DNaseI, extracted with phenol
5 followed chloroform:isoamyl alcohol (25:1), precipitated with isopropanol and washed with 70% ethanol. The dried pellet was resuspended in 20 μ l DEPC-treated water and stored at -20°C.

Unlabeled ribozyme (200 nM) and internally labeled 1.8 KB substrate RNA (<10 nM) were denatured and renatured separately in a standard cleavage buffer (containing 50 mM Tris-HCl pH 7.5 and 10 mM MgCl₂) by
10 heating to 90°C for 2 min. and slow cooling to 37°C for 10 min. The reaction was initiated by mixing the ribozyme and substrate mixtures and incubating at 37°C. Aliquots of 5 μ l were taken at regular time intervals, quenched by adding an equal volume of 2X formamide gel loading buffer and frozen on dry
15 ice. The samples were resolved on 5% polyacrylamide sequencing gel and results were quantitatively analyzed by radioanalytic imaging of gels with a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

Few antiviral drug therapies are available that effectively inhibit established viral infections. Consequently, prophylactic immunization has
20 become the method of choice for protection against viral pathogens. However, effective vaccines for divergent viruses such as those causing the common cold, and HIV, the etiologic agent of AIDS, may not be feasible. Consequently, new antiviral strategies are being developed for combating viral infections.

Gene therapy represents a potential alternative strategy, where antiviral genes are stably transferred into susceptible cells. Such gene therapy approaches have been termed "intracellular immunization" since cells expressing antiviral genes become immune to viral infection (Baltimore, 1988
25 *Nature* 335, 395-396). Numerous forms of antiviral genes have been developed, including protein-based antivirals such as transdominant inhibitory proteins (Malim et al., 1993 *J. Exp. Med.*, Bevec et al., 1992 *P.N.A.S. (USA)*
30 89, 9870-9874; Bahner et al., 1993 *J. Virol.* 67, 3199-3207) and viral-activated suicide genes (Ashorn et al., 1990 *P.N.A.S.(USA)* 87, 8889-8893). Although

effective in tissue culture, protein-based antivirals have the potential to be immunogenic *in vivo*. It is therefore conceivable that treated cells expressing such foreign antiviral proteins will be eradicated by normal immune functions. Alternatives to protein based antivirals are RNA based molecules such as antisense RNAs, decoy RNAs, agonist RNAs, antagonist RNAs, therapeutic editing RNAs and ribozymes. RNA is not immunogenic; therefore, cells expressing such therapeutic RNAs are not susceptible to immune eradication.

Example 90: Design and construction of U6-S35 Chimera

A transcription unit, termed **U6-S35**, is designed that contains the characteristic intramolecular stem of a **S35** motif (see Figure 76). As shown in Figure 77, 78 and 79 a desired RNA (e.g. ribozyme) can be inserted into the indicated region of U6-S35 chimera. This construct is under the control of a type 3 pol III promoter, such as a mammalian U6 small nuclear RNA (snRNA) promoter (see Fig. 75). U6-S35-HHI and U6-S35-HHII are non-limiting examples of the U6-S35 chimera.

As a non-limiting example, applicant has constructed a stable, active ribozyme RNA driven from a eukaryotic U6 promoter (Fig. 78). For stability, applicant incorporated a S35 motif as described in Fig. 76 and Fig. 77. A ribozyme sequence is inserted at the top of the stem, such that the ribozyme is separated from the S35 motif by an unstructured spacer sequence (Fig. 77, 78, 79). The spacer sequence can be customized for each desired RNA sequence. U6-S35 chimera is meant to be a non-limiting example and those skilled in the art will recognize that the structure disclosed in the figures 77, 78 and 79 can be driven by any of the known RNA polymerase promoters and are within the scope of this invention. All that is necessary is for the 5' region of a transcript to interact with its 3' region to form a stable intramolecular structure (S35 motif) and that the S35 motif is separated from the desired RNA by a stretch of unstructured spacer sequence. The spacer sequence appears to improve the effectiveness of the desired RNA.

By "unstructured" is meant lack of a secondary and tertiary structure such as lack of any stable base-paired structure within the sequence itself, and preferably with other sequences in the attached RNA.

By "spacer sequence" is meant any unstructured RNA sequence that separates the S35 domain from the desired RNA. The spacer sequence can be greater than or equal to one nucleotide.

In vitro Catalytic Activity of U6-S35-Ribozyme Chimeras:

- 5 U6-S35-HHI ribozyme RNA was synthesized using T7 RNA polymerase. HHI RNA was chemically synthesized using RNA phosphoramidite chemistry as described in Wincott et al., 1995 *Nucleic Acids Res.* The ribozyme RNAs were gel-purified and the purified ribozyme RNAs were heated to 55°C for 5 min. Target RNA used was ~650 nucleotide long. Internally-³²P-labeled
- 10 target RNA was prepared as described above. The target RNA was pre-heated to 37°C in 50 mM Tris.HCl, 10 mM MgCl₂ and then mixed at time zero with the ribozyme RNAs (to give 200 nM final concentration of ribozyme). At appropriate times an aliquot was removed and the reaction was stopped by dilution in 95% formamide. Samples were resolved on a denaturing urea-
- 15 polyacrylamide gel and products were quantitated on a phosphorimager®.

As shown in Figure 80, the U6-S35-HHI ribozyme chimera cleaved its target RNA as efficiently as a chemically synthesized HHI ribozyme. In fact, it appears that the U6-S35-HHI ribozyme chimera may be more efficient than the synthetic ribozyme.

20 *Accumulation of U6-S35-ribozyme transcripts*

- An Actinomycin D assay was used to measure accumulation of the transcript in mammalian cells. Cells were transfected overnight with plasmids encoding the appropriate transcription units (2µg DNA/well of 6 well plate) using calcium phosphate precipitation method (Maniatis et al., 1982 *Molecular*
- 25 *Cloning* Cold Spring Harbor Laboratory Press, NY). After the overnight transfection, media was replaced and the cells were incubated an additional 24 hours. Cells were then incubated in media containing 5µg/ml Actinomycin D. At the times indicated, cells were lysed in guanidinium isothiocyanate, and total RNA was purified by phenol/chloroform extraction and isopropanol
- 30 precipitation as described by Chomczynski and Sacchi, 1987 *Anal. Biochem.*, 162, 156. RNA was analyzed by northern blot analysis and the levels of

specific RNAs were radioanalytically quantitated on a phosphorimager[®]. The level of RNA at time zero was set to be 100%.

As shown in Figure 81, the U6-S35-HHII ribozyme shown in Figure 79 is fairly stable in 293 mammalian cells with an approximate half-life of about 2
5 hours.

Example 91: Design and construction of VA1-S35 Chimera

Referring to Figure 83A, In order to express ribozymes from a VA1 promoter, applicant has constructed a transcription unit consisting of a wild type VA1 sequence with two modifications: a "S35-like" motif extends from a
10 loop in the central domain (Figure 82); the 3' terminus is changed such that there is a more complete interaction between the 5' and the 3' region of the transcript (specifically, an "A-C" bulge is changed to an "A-U base pair and the termination sequence is part of the stem of S35 motif).

Accumulation of VA1-S35-ribozyme transcripts

15 An Actinomycin D assay was used to measure accumulation of the transcript in mammalian cells as described above. As shown in Figure 84, the VA1-S35-chimera, shown in Figure 83A, has approximately 10-fold higher stability in 293 mammalian cells compared to VA1-chimera, shown in Figure 25B that lacks the intramolecular S35 motif.

20 Besides ribozymes, desired RNAs like antisense, therapeutic editing RNAs, decoys, can be readily inserted into the indicated U6-S35 or VA1-S35 chimera to achieve therapeutic levels of RNA expression in mammalian cells.

Sequences listed in the Figures are meant to be non-limiting examples. Those skilled in the art will recognize that variants (mutations, insertions and
25 deletions) of the above examples can be readily generated using techniques known in the art, are within the scope of the present invention.

Diagnostic uses

Ribozymes of this invention may be used as diagnostic tools to examine genetic drift and mutations within diseased cells or to detect the presence of

stromolysin, B7-1, B7-2, B7-3 and/or CD40 or other RNAs in a cell. The close relationship between ribozyme activity and the structure of the target RNA allows the detection of mutations in any region of the molecule which alters the base-pairing and three-dimensional structure of the target RNA. By using multiple ribozymes described in this invention, one may map nucleotide changes which are important to RNA structure and function *in vitro*, as well as in cells and tissues. Cleavage of target RNAs with ribozymes may be used to inhibit gene expression and define the role (essentially) of specified gene products in the progression of disease. In this manner, other genetic targets may be defined as important mediators of the disease. These experiments will lead to better treatment of the disease progression by affording the possibility of combinational therapies (e.g., multiple ribozymes targeted to different genes, ribozymes coupled with known small molecule inhibitors, or intermittent treatment with combinations of ribozymes and/or other chemical or biological molecules). Other *in vitro* uses of ribozymes of this invention are well known in the art, and include detection of the presence of mRNAs associated with B7-1, B7-2, B7-3 and/or CD40 or other RNA related conditions. Such RNA is detected by determining the presence of a cleavage product after treatment with a ribozyme using standard methodology.

In a specific example, ribozymes which can cleave only wild-type or mutant forms of the target RNA are used for the assay. The first ribozyme is used to identify wild-type RNA present in the sample and the second ribozyme will be used to identify mutant RNA in the sample. As reaction controls, synthetic substrates of both wild-type and mutant RNA will be cleaved by both ribozymes to demonstrate the relative ribozyme efficiencies in the reactions and the absence of cleavage of the "non-targeted" RNA species. The cleavage products from the synthetic substrates will also serve to generate size markers for the analysis of wild-type and mutant RNAs in the sample population. Thus each analysis will require two ribozymes, two substrates and one unknown sample which will be combined into six reactions. The presence of cleavage products will be determined using an RNase protection assay so that full-length and cleavage fragments of each RNA can be analyzed in one lane of a polyacrylamide gel. It is not absolutely required to quantify the results to gain insight into the expression of mutant RNAs and

- putative risk of the desired phenotypic changes in target cells. The expression of mRNA whose protein product is implicated in the development of the phenotype (*i.e.*, B7-1, B7-2, B7-3 and/or CD40) is adequate to establish risk. If probes of comparable specific activity are used for both transcripts, then a
- 5 qualitative comparison of RNA levels will be adequate and will decrease the cost of the initial diagnosis. Higher mutant form to wild-type ratios will be correlated with higher risk whether RNA levels are compared qualitatively or quantitatively.

Other embodiments are within the following claims.

TABLE ICharacteristics of Ribozymes**Group I Introns**

Size: ~200 to >1000 nucleotides.

Requires a U in the target sequence immediately 5' of the cleavage site.

Binds 4-6 nucleotides at 5' side of cleavage site.

Over 75 known members of this class. Found in *Tetrahymena thermophila* rRNA, fungal mitochondria, chloroplasts, phage T4, blue-green algae, and others.

RNAseP RNA (M1 RNA)

Size: ~290 to 400 nucleotides.

RNA portion of a ribonucleoprotein enzyme. Cleaves tRNA precursors to form mature tRNA.

Roughly 10 known members of this group all are bacterial in origin.

Hammerhead Ribozyme

Size: ~13 to 40 nucleotides.

Requires the target sequence UH immediately 5' of the cleavage site.

Binds a variable number nucleotides on both sides of the cleavage site.

14 known members of this class. Found in a number of plant pathogens (virusoids) that use RNA as the infectious agent (Figure 1)

HaIrpin Ribozyme

Size: ~50 nucleotides.

Requires the target sequence GUC immediately 3' of the cleavage site.

Binds 4-6 nucleotides at 5' side of the cleavage site and a variable number to the 3' side of the cleavage site.

Only 3 known member of this class. Found in three plant pathogen (satellite RNAs of the tobacco ringspot virus, arabis mosaic virus and chicory yellow mottle virus) which uses RNA as the infectious agent (Figure 3).

Hepatitis Delta Virus (HDV) Ribozyme

Size: 50 - 60 nucleotides (at present).

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined, although no sequences 5' of cleavage site are required.

Only 1 known member of this class. Found in human HDV (Figure 4).

***Neurospora* VS RNA Ribozyme**

Size: ~144 nucleotides (at present)

Cleavage of target RNAs recently demonstrated.

Sequence requirements not fully determined.

Binding sites and structural requirements not fully determined. Only 1 known member of this class. Found in *Neurospora* VS RNA (Figure 5).

Table AII: Human Stromelysin Hammerhead Target Sequence

<u>nt</u> <u>Position</u>	<u>Sequence</u>	<u>SEQ. ID. NO.</u>
20	UAGAGCUAAGUAAAGCCAG	ID. NO. 01
126	ACAACCAGCAUGAA	ID. NO. 02
147	AGAAAUUUCUAGA	ID. NO. 03
171	ACCUCAAAAAGAUGUGAAACAGU	ID. NO. 04
240	AAAUCCAGAAGUUC	ID. NO. 05
287	GACACUCUGGAGGUGAUGCGCAAGCCCAGGUGU	ID. NO. 06
327	CUGAUGUUGGUCACUUCAGAAC	ID. NO. 07
357	GCAUCCCGAAGUGGAGGAAAACCCACCUUACAU	ID. NO. 08
402	AUUUAUACACCAGAUUUGCCAAAAGAUG	ID. NO. 09
429	CUGUUGAUUCUGCUGUGAGA	ID. NO. 10
455	CUGAAAGUCUGGGAAGAGGUGA	ID. NO. 11
513	CUGAUUAUAUGA	ID. NO. 12
592	UGCCU AUGCCCC	ID. NO. 13
624	AUGCCCACUUUGAUGAUGAUGAACAAUGGACA	ID. NO. 14
679	AUUUCUUGUUGCUGCUCAUG	ID. NO. 15
725	CACUCAGCCAACACUGA	ID. NO. 16
801	AAGAUGAUUAUAAUUGCAUUCAGUCC	ID. NO. 17
827	CUCUAUGGACCUCCCCUGACUCCCCU	ID. NO. 18
859	CCCCUGGUACCCA	ID. NO. 19
916	UCCUGCUUUGUCCUUGAUGCUGUCAGCAC	ID. NO. 20
958	AAUCCUGAUUUUAAAGA	ID. NO. 21
975	CAGGCACUUUUGGCGCAAUCCC	ID. NO. 22
1018	AUUGCAUUUGAUCUCUUCAUUUUGGCCAUC	ID. NO. 23
1070	GCAUAUGAAGUUA	ID. NO. 24
1203	AAAUUGAUGCAGCCAUUUCUGA	ID. NO. 25
1274	UUUGAUGAGAAGAGAAAUUCCAUGGAGC	ID. NO. 26
1302	CAGGCUUCCCAAGCAAUAGCUGAAGAC	ID. NO. 27
1420	CCCAAUGCAAAG	ID. NO. 28
1485	AUGUAGAAGGCACAUAUUGGCACUUUAAA	ID. NO. 29
1623	UCUUGCCGGUCAUUUUUAUGUUAU	ID. NO. 30
1665	GCUGCUGCUUAGC	ID. NO. 31

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1733

CAACAGACAAGUGACUGUAUCU

ID. NO. 32

1769

CUUAUUUAUA

ID. NO. 33

Table AIII: Human Stromelysin HH Target Sequence

nt. Position	Target Sequence	Seq. ID. NO.
10	GCAAGGCAUA GAGACAACAUAGAGC	ID. NO. 34
21	GCAUAGAGACAACAU GAGCUAAGUAAAGCC	ID. NO. 35
27	AGACAACAUAGAGCUA AGUAAAGCCAGUGGA	ID. NO. 36
31	AACAUAGAGCUAAGUA AAGCCAGUGGAAAU	ID. NO. 37
53	GUGGAAAUAGAGAGUC UUCCA AUCCUACUGU	ID. NO. 38
55	GGAAUAGAGAGUCUU CCAUCCUACUGUUG	ID. NO. 39
56	GAAUAGAGAGUCUUC CAUCCUACUGUUGC	ID. NO. 40
61	GAAGAGUCUCCAUC CUACUGUUGCUGUC	ID. NO. 41
64	GAGUCUCCA AUCCUA CUGUUGCUGUGUG	ID. NO. 42
69	UUCCA AUCCUACUGU GCUGUGUGGCGAGU	ID. NO. 43
85	GCUGUGUGGCGAGU UGCUCAGCCUAUCCA	ID. NO. 44
86	CUGUGUGGCGAGUU GCUCAGCCUAUCCAU	ID. NO. 45
90	GUGUGGCGAGUUGCUC AGCCUAUCCAUUGGA	ID. NO. 46
96	CAGUUUGCUCAGCCUA UCCAUUGGAUGGAGC	ID. NO. 47
98	GUUUGCUCAGCCUAC CAUUGGAGGAGCUG	ID. NO. 48
102	GCUCAGCCUAUCCAU GGAUGGAGCUGCAAG	ID. NO. 49
142	CACCAGCAUGAACCUU GUUCAGAAAUUCUA	ID. NO. 50
145	CAGCAUGAACCUUGU CAGAAAUUCUAGAA	ID. NO. 51
146	AGCAUGAACCUUGUC AGAAAUUCUAGAAA	ID. NO. 52
153	ACCUUGUUCAGAAUA UCUAGAAAACUACUA	ID. NO. 53
155	CUUGUUCAGAAUAUC UAGAAAACUACUACG	ID. NO. 54
157	UGUUCAGAAAUUCUA GAAAACUACUACGAC	ID. NO. 55
165	AAUAUCUAGAAAACUA CUACGACCUCAAAAA	ID. NO. 56
168	AUCUAGAAAACUACUA CGACCUCAAAAAAGA	ID. NO. 57
175	AAACUACUACGACCUC AAAAAAGAUUGUGAAA	ID. NO. 58
195	AAGAUGUGAAACAGUU UGUUAGGAGAAAGGA	ID. NO. 59
196	AGAUGUGAAACAGUUU GUUAGGAGAAAGGAC	ID. NO. 60

199	UGUGAAACAGUUGUU AGGAGAAAGGACAGU	ID. NO. 61
200	GUGAAACAGUUGUUA GGAGAAAGGACAGUG	ID. NO. 62
218	AGAAAGGACAGUGGUC CUGUUGUUAATAAAA	ID. NO. 63
223	GGACAGUGGUCCUGU GUUAAAAAAUCCGA	ID. NO. 64
226	CAGUGGUCCUGUUGU AAAAAAUCCGAGAA	ID. NO. 65
227	AGUGGUCCUGUUGUA AAAAAUCCGAGAAA	ID. NO. 66
235	UGUUGUUAATAAAUC CGAGAAUUCGAGAAG	ID. NO. 67
252	GAGAAUUCGAGAAGU CCUUGGUAUGGAGGU	ID. NO. 68
253	AGAAUUCGAGAAGUUC CUUGGAUUGGAGGUG	ID. NO. 69
256	AAUUCGAGAAGUCCU GGAUUGGAGGUGAGC	ID. NO. 70
261	AGAAGUCCUUGGAU GGAGGUGAGCGGGAA	ID. NO. 71
285	CGGGGAAGCUGGACUC CGACACUCUGGAGGU	ID. NO. 72
293	CUGGACUCCGACACUC UGGAGGUGAUGGCA	ID. NO. 73
325	GCCCAGGUGUGGAGU CCUGAUGUUGGUCAC	ID. NO. 74
326	CCCAGGUGUGGAGUUC CUGAUGUUGGUCACU	ID. NO. 75
334	UGGAGUCCUGAUGU GGUCACUUCAGAACC	ID. NO. 76
338	GUCCUGAUGUUGGUC ACUUCAGAACCUUC	ID. NO. 77
342	CUGAUGUUGGUCACU CAGAACCUUCCUGG	ID. NO. 78
343	UGAUGUUGGUCACUC AGAACCUUCCUGGC	ID. NO. 79
351	GUCACUUCAGAACCUU UCCUGGCAUCCCGAA	ID. NO. 80
352	UCACUUCAGAACCUU CCUGGCAUCCCGAAG	ID. NO. 81
353	CACUUCAGAACCUUC CUGGCAUCCCGAAGU	ID. NO. 82
361	AACCUUCCUGGCAUC CCGAAGUGGAGGAAA	ID. NO. 83
385	GAGGAAAACCCACCUU ACUAACAGGAUUGUG	ID. NO. 84
386	AGGAAAACCCACCUUA CAUAACAGGAUUGUGA	ID. NO. 85
390	AAACCCACCUUACAUA CAGGAUUGUGAAUUA	ID. NO. 86
397	CCUUAUAACAGGAU GUGAAUUAUACCA	ID. NO. 87
404	UACAGGAUUGUGAAU AUACACCAGAUUUGC	ID. NO. 88
405	ACAGGAUUGUGAAUUA UACACCAGAUUUGCC	ID. NO. 89
407	AGGAUUGUGAAUUA CACCAGAUUUGCCAA	ID. NO. 90
416	AAUUAUAACACAGAU UGCCAAAAGAUGCUG	ID. NO. 91
417	AUUAUAACACAGAUU GCCAAAAGAUGCUGU	ID. NO. 92
433	GCCAAAAGAUGCUGU GAUUCUGCUGUUGAG	ID. NO. 93
437	AAAGAUGCUGUUGAU CUGCUGUUGAGAAAG	ID. NO. 94
438	AAGAUGCUGUUGAUUC UGCUGUUGAGAAAGC	ID. NO. 95
445	UGUUGAUUCUGCUGU GAGAAAGCUCUGAAA	ID. NO. 96

455	GCUGUUGAGAAAGCUC UGAAAGUCUGGGAAG	ID. NO. 97
463	GAAAGCUCUGAAAGUC UGGGAAGAGGUGACU	ID. NO. 98
479	UGGGAAGAGGUGACUC CACUCACAUUCUCCA	ID. NO. 99
484	AGAGGUGACUCCACUC ACAUUCUCCAGGCUG	ID. NO. 100
489	UGACUCCACUCACAUU CUCCAGGCUGUAUGA	ID. NO. 101
490	GACUCCACUCACAUUC UCCAGGCUGUAUGAA	ID. NO. 102
492	CUCCACUCACAUUCUC CAGGCUGUAUGAAGG	ID. NO. 103
501	CAUUCUCCAGGCUGUA UGAAGGAGAGGCUGA	ID. NO. 104
518	GAAGGAGAGGCUGAUA UAAUGAUCUCUUUG	ID. NO. 105
520	AGGAGAGGCUGAUAUA AUGAUCUCUUUGCA	ID. NO. 106
526	GGCUGAUAUAUGAUC UCUUUUGCAGUAGA	ID. NO. 107
528	CUGAUAUAUGAUCUC UUUUGCAGUAGAGA	ID. NO. 108
530	GUAUAUAUGAUCUCU UUGCAGUAGAGAAC	ID. NO. 109
531	AUAUAUAUGAUCUCUU UGCAGUAGAGACA	ID. NO. 110
532	UAUAUAUGAUCUCUUU GCAGUAGAGAACAU	ID. NO. 111
538	GAUCUCUUUUGCAGUU AGAGAACAUGGAGAC	ID. NO. 112
539	AUCUCUUUUGCAGUA GAGAACAUGGAGACU	ID. NO. 113
555	GAGAACAUGGAGACUU UUAOCUUUUGAUGG	ID. NO. 114
556	AGAACAUGGAGACUUU UACOCUUUUGAUGGA	ID. NO. 115
557	GAACAUGGAGACUUU AOCUUUUGAUGGAC	ID. NO. 116
558	AACAUGGAGACUUUA OCCUUUUGAUGGACC	ID. NO. 117
563	GGAGACUUUAOCUUU UUGAUGGACCUGGAA	ID. NO. 118
564	GAGACUUUAOCUUU UGAUGGACCUGGAAA	ID. NO. 119
565	AGACUUUAOCUUUU GAUGGACCUGGAAAU	ID. NO. 120
583	UGGACCUGGAAUGUU UUGGCCAUGCCUAU	ID. NO. 121
584	GGACCUGGAAUGUUU UGGCCAUGCCUAUG	ID. NO. 122
585	GACCUGGAAUGUUUU GGCCAUGCCUAUGC	ID. NO. 123
597	UUUUGGCCAUGCCUA UGCCCCUGGGCCAGG	ID. NO. 124
616	CCUUGGGCCAGGGAUU AAUGGAGAUGCCAC	ID. NO. 125
617	CCUGGGCCAGGGAUA AUGGAGAUGCCACU	ID. NO. 126
633	AUGGAGAUGCCACUU UGAUGAUGAUGAACA	ID. NO. 127
634	UGGAGAUGCCACUUU GAUGAUGAUGAACAA	ID. NO. 128
662	CAAUGGACAAAGGAUA CAACAGGGACCAAUU	ID. NO. 129
677	ACAACAGGGACCAAUU UAUUUCUGUUGCUG	ID. NO. 130
678	CAACAGGGACCAAUUU AUUUUCUGUUGCUGC	ID. NO. 131
679	AACAGGGACCAAUUA UUUUCUGUUGCUGCU	ID. NO. 132

681	CAGGGACCAAUUUAUU UCUUGUUGCUGCUCA	ID. NO. 133
682	AGGGACCAAUUUAUU CUUGUUGCUGCUCAU	ID. NO. 134
683	GGGACCAAUUUAUUUC UCGUUGCUGCUCAUG	ID. NO. 135
685	GACCAAUUUAUUUCUC GUUGCUGCUCAUGAA	ID. NO. 136
688	CAAUUUAUUUCUGUU GCUGCUCAUGAAAU	ID. NO. 137
695	UUUCUGUUGCUGCUC AUGAAAUUGGOCACU	ID. NO. 138
703	UGCUGCUCAUGAAAUU GGCCACUCCUGGGU	ID. NO. 139
711	AUGAAAUUGGOCACUC CCUGGGUCUCUUCA	ID. NO. 140
719	GGCCACUCCUGGGUC UCUUUCACUCAGCCA	ID. NO. 141
721	CCACUCCUGGGUCUC UUUCACUCAGCCAAC	ID. NO. 142
723	ACUCCUGGGUCUCUU UCACUCAGCCAACAC	ID. NO. 143
724	CUCCUGGGUCUCUUU CACUCAGCCAACACU	ID. NO. 144
725	UCCUGGGUCUCUUUC ACUCAGCCAACACUG	ID. NO. 145
729	UGGGUCUCUUUCACUC AGCCAACACUGAAGC	ID. NO. 146
746	GCCAACACUGAAGCUU UGAUGUACCCACUCU	ID. NO. 147
747	CCAACACUGAAGCUUU GAUGUACCCACUCUA	ID. NO. 148
753	CUGAAGCUUUGAUGUA CCCACUCUAUCACUC	ID. NO. 149
760	UUUGAUGUACCCACUC UAUCACUCACUCACA	ID. NO. 150
762	UGAUGUACCCACUCUA UCACUCACUCACAGA	ID. NO. 151
764	AUGUACCCACUCUAUC ACUCACUCACAGACC	ID. NO. 152
768	ACCCACUCUAUCACUC ACUCACAGACCUGAC	ID. NO. 153
772	ACUCUAUCACUCACUC ACAGACCUGACUCCG	ID. NO. 154
785	CUCACAGACCUGACUC GGUUCCGCCUGUCUC	ID. NO. 155
789	CAGACCUGACUCCGUU CCGCCUGUCUCAAGA	ID. NO. 156
790	AGACCUGACUCCGUUC CGCCUGUCUCAAGAU	ID. NO. 157
798	CUCCGUUCCGCCUGUC UCAAGAUGAUUAAA	ID. NO. 158
800	CCGUUCCGCCUGUCUC AAGAUGAUUAAUUG	ID. NO. 159
809	CUGUCUCAAGAUGAUA UAAUUGGCAUUCAGU	ID. NO. 160
811	GUCUCAAGAUGAUAUA AAUGGCAUUCAGUCC	ID. NO. 161
820	UGAUAUAUAAUUGGCAUU CAGUCCCUUAUGGA	ID. NO. 162
821	GAUAUAUAAUUGGCAUUC AGUCCCUUAUGGAC	ID. NO. 163
825	UAAUUGGCAUUCAGUC CCUCUAUGGACCUCC	ID. NO. 164
829	UGGCAUUCAGUCCUUC UAUGGACCUCCCCCU	ID. NO. 165
831	GCAUUCAGUCCUCUA UGGACCUCCCCCUGA	ID. NO. 166
839	UCCUCUAUGGACUUC CCCCUGACUCCUCCG	ID. NO. 167
849	GACCUCCCCCUGACUC CCCCUGAGACCCCCCU	ID. NO. 168

868	UGAGACCCCCUGGUA CCCACGGAAACUGUC	ID. NO. 169
883	AOCCACGGAAACUGUC CCUOCAGAAACUGGG	ID. NO. 170
887	AOGGAACUGUCCCC CAGAACUUGGGAGGC	ID. NO. 171
917	CCAGCCAACUGUGAUC CUGCUUUGUCCUUUG	ID. NO. 172
923	AACUGUGAUCCUGCUU UGUCCUUUGAUGCUG	ID. NO. 173
924	ACUGUGAUCCUGCUUU GUCCUUUGAUGCUGU	ID. NO. 174
927	GUGAUCCUGCUUUGUC CUUUGAUGCUGUCAG	ID. NO. 175
930	AUCCUGCUUUGUCCUU UGAUGCUGUCAGCAC	ID. NO. 176
931	UCCUGCUUUGUCCUU GAUGCUGUCAGCACU	ID. NO. 177
940	GUCCUUUGAUGCUGUC AGCACUCUGAGGGGA	ID. NO. 178
947	GAUGCUGUCAGCACUC UGAGGGGAGAAUCC	ID. NO. 179
961	UCUGAGGGGAGAAUUC CUGAUCUUUAAGAC	ID. NO. 180
967	GGGAGAAAUCCUGAUC UUAAGACAGGCAC	ID. NO. 181
969	GAGAAAUCCUGAUCUU UAAAGACAGGCACUU	ID. NO. 182
970	AGAAAUCCUGAUCUU AAAGACAGGCACUUU	ID. NO. 183
971	GAAAUCCUGAUCUUUA AAGACAGGCACUUU	ID. NO. 184
984	UUAAGACAGGCACUU UUGGCGCAAUCCCU	ID. NO. 185
985	UAAAGACAGGCACUUU UGGCGCAAUCCUC	ID. NO. 186
986	AAAGACAGGCACUUU GGGCAAUCCCUCA	ID. NO. 187
996	ACUUUUGGCGCAAUUC CCUCAGGAAGCUUGA	ID. NO. 188
1000	UUGGCGCAAUCCUC AGGAAGCUUGAACCU	ID. NO. 189
1009	AUCCUCAGGAAGCUU GAACUGAAUUGCAU	ID. NO. 190
1020	AGCUUGAACCUUGAUA GCAUUGAUCUCUUC	ID. NO. 191
1025	GAAACUGAAUUGCAU UGAUCUCUUCAUUU	ID. NO. 192
1026	AACCUGAAUUGCAUU GAUCUCUUCAUUUUG	ID. NO. 193
1030	UGAAUUGCAUUGAUC UCUUCAUUUUGGCA	ID. NO. 194
1032	AAUUGCAUUGAUCUC UUCAUUUUGGCCAUC	ID. NO. 195
1034	UUGCAUUGAUCUCU CAUUUUGGCCAUCUC	ID. NO. 196
1035	UGCAUUGAUCUCUC AUUUUGGCCAUCUCU	ID. NO. 197
1038	AUUUGAUCUCUUCAU UUGGCCAUCUCUCC	ID. NO. 198
1039	UUUGAUCUCUUCAUU UGGCCAUCUCUCCU	ID. NO. 199
1040	UUGAUCUCUUCAUUU GGCCAUCUCUCCU	ID. NO. 200
1047	CUUCAUUUUGGCCAUC UCUCCUUCAGGCGU	ID. NO. 201
1049	UCAUUUUGGCCAUCUC UUCUUCAGGCGUGG	ID. NO. 202
1051	AUUUUGGCCAUCUCU CCUUCAGGCGUGGAU	ID. NO. 203
1052	UUUUGGCCAUCUCUC CUUCAGGCGUGGAUG	ID. NO. 204

1055	UGGCCAUCUCUUCUU CAGGCGUGGAUGCCG	ID. NO. 205
1056	GGCCAUUCUCUUCUUC AGGCGUGGAUGCCG	ID. NO. 206
1074	GCGUGGAUGCCGCAUA UGAAGUUAUAAGCAA	ID. NO. 207
1081	UGCCGCAUAUGAAGUU ACUAGCAAGGACCUC	ID. NO. 208
1082	GCCGCAUAUGAAGUUA CUAGCAAGGACCUC	ID. NO. 209
1085	GCAUAUGAAGUUAUA GCAAGGACCUCGUUU	ID. NO. 210
1096	UACUAGCAAGGACCUC GUUUCAUUUUUAAA	ID. NO. 211
1099	UAGCAAGGACCUCGUU UUCAUUUUUAAAGGA	ID. NO. 212
1100	AGCAAGGACCUCGUUU UCAUUUUUAAAGGAA	ID. NO. 213
1101	GCAAGGACCUCGUUU CAUUUUUAAAGGAAA	ID. NO. 214
1102	CAAGGACCUCGUUUU AUUUUUAAAGGAAU	ID. NO. 215
1105	GGACCUUGUUUCAU UUUAAAGGAAAUCAA	ID. NO. 216
1106	GACCUUGUUUCAUUU UUAAGGAAAUCAAU	ID. NO. 217
1107	ACCUUGUUUCAUUUU UAAAGGAAAUCAAU	ID. NO. 218
1108	CCUUGUUUCAUUUU AAAGGAAAUCAAUC	ID. NO. 219
1109	CUUGUUUCAUUUUUA AAGGAAAUCAAUCU	ID. NO. 220
1118	AUUUUUAAAGGAAUC AAUUCUGGGCCAUA	ID. NO. 221
1122	UUAAAGGAAAUCAAU CUGGGCCAUCAGAGG	ID. NO. 222
1123	UAAAGGAAAUCAAUC UGGGCCAUCAGAGGA	ID. NO. 223
1132	UCAAUUCUGGGCCAUC AGAGGAAAUAGAGUA	ID. NO. 224
1147	CAGAGGAAAUAGAGUA CGAGCUGGAUACCCA	ID. NO. 225
1158	AGGUACGAGCUGGUA CCCAAGAGGCAUCCA	ID. NO. 226
1171	AUAACCAAGAGGCAUC CACACCCUAGGUUC	ID. NO. 227
1180	AGGCAUCCACACCCUA GGUUCCCCUCCAACC	ID. NO. 228
1184	AUCCACACCCUAGGUU UCCCUCCAACCGUGA	ID. NO. 229
1185	UCCACACCCUAGGUU CCCCUCACACCGUGAG	ID. NO. 230
1186	CCACACCCUAGGUUC CCUCCAACCGUGAGG	ID. NO. 231
1190	ACCCUAGGUUCCUC CAACCGUGAGGAAAA	ID. NO. 232
1207	AACCGUGAGGAAAUUC GAUGCAGCCAUUCU	ID. NO. 233
1219	AAUGAUGCAGCCAUU UCUGAUAAGGAAAAG	ID. NO. 234
1220	AUGAUGCAGCCAUUU CUGAUAAGGAAAAGA	ID. NO. 235
1221	UUGAUGCAGCCAUUC UGAUAAGGAAAAGAA	ID. NO. 236
1226	GCAGCCAUUUCUGAUA AGGAAAAGAACAAA	ID. NO. 237
1245	AAAAGAACAAAACALIA UUUCUUGUAGAGGA	ID. NO. 238
1247	AAGAACAAAACAUUU UCUCUUGUAGAGGACA	ID. NO. 239
1248	AGAACAAAACAUUUU CUUUGUAGAGGACAA	ID. NO. 240

1249	GAACAAAACAUUUUC UUUGUAGAGGACAAA	ID. NO. 241
1251	ACAAAACAUUUUCUU UGUAGAGGACAAUA	ID. NO. 242
1252	CAAAACAUUUUCUUU GUAGAGGACAAUAC	ID. NO. 243
1255	AACAUUUUCUUUGUA GAGGACAAUACUGG	ID. NO. 244
1266	UUUGUAGAGGACAAUA CUGGAGAUUUGAUGA	ID. NO. 245
1275	ACAAUACUGGAGAUU UGAUGAGAGAGAAA	ID. NO. 246
1276	CAAUACUGGAGAUUU GAUGAGAAGAGAAU	ID. NO. 247
1292	GAUGAGAAGAGAAAU CCAUGGAGCCAGGCU	ID. NO. 248
1293	AUGAGAAGAGAAUUC CAUGGAGCCAGGCUU	ID. NO. 249
1308	CCAUGGAGCCAGGCUU UCCCAAGCAAUAGC	ID. NO. 250
1309	CAUGGAGCCAGGCUUU CCCAAGCAAUAGCU	ID. NO. 251
1310	AUGGAGCCAGGCUUC CCAAGCAAUAGCUG	ID. NO. 252
1321	CUUCCCCAAGCAAUA GCUGAAGACUUUCCA	ID. NO. 253
1332	AAAUAGCUGAAGACUU UCCAGGGAUUGACUC	ID. NO. 254
1333	AAUAGCUGAAGACUUU CCAGGGAUUGACUCA	ID. NO. 255
1334	AUAGCUGAAGACUUUC CAGGGAUUGACUCA	ID. NO. 256
1342	AGACUUUCCAGGGAUU GACUCAAGAUUGAU	ID. NO. 257
1347	UUCAGGGAUUGACUC AAAGAUUGAUGCUGU	ID. NO. 258
1354	GAUUGACUCAAGAUU GAUGCUGUUUUGAA	ID. NO. 259
1363	AAAGAUUGAUGCUGUU UUUGAAGAAUUGGG	ID. NO. 260
1364	AAGAUUGAUGCUGUUU UUGAAGAAUUGGGU	ID. NO. 261
1365	AGAUUGAUGCUGUUU UGAAGAAUUGGGUU	ID. NO. 262
1366	GAUUGAUGCUGUUUU GAAGAAUUGGGUUC	ID. NO. 263
1374	CUGUUUUUGAAGAAU UGGGUUCUUUUAUU	ID. NO. 264
1375	UGUUUUUGAAGAAUU GGGUUCUUUUAUUC	ID. NO. 265
1380	UUGAAGAAUUGGGUU CUUUUAUUCUUUAC	ID. NO. 266
1381	UGAAGAAUUGGGUUC UUUUAUUCUUUACU	ID. NO. 267
1383	AAGAAUUGGGUUCUU UUAUUUCUUUACUGG	ID. NO. 268
1384	AGAAUUGGGUUCUUU UAUUUUUUACUGGA	ID. NO. 269
1385	GAAUUGGGUUCUUUU AUUUCUUUACUGGAU	ID. NO. 270
1386	AAUUGGGUUCUUUUA UUUCUUUACUGGALC	ID. NO. 271
1388	UUUGGGUUCUUUAUU UCUUUACUGGAUCUU	ID. NO. 272
1389	UUGGGUUCUUUAUUU CUUUACUGGAUCUUC	ID. NO. 273
1390	UGGGUUCUUUAUUUC UUUACUGGAUCUACA	ID. NO. 274
1392	GGUUCUUUAUUUCUU UACUGGAUCUUCACA	ID. NO. 275
1393	GUUCUUUAUUUCUUU ACUGGAUCUUCACAG	ID. NO. 276

1394	UUCUUUUAUUCUUUA CUGGAUCUUCACAGU	ID. NO. 277
1401	AUUUCUUUACUGGauc UUCACAGUUGGAGUU	ID. NO. 278
1403	UUCUUUACUGGAUCUU CACAGUUGGAGUUUG	ID. NO. 279
1404	UCUUUACUGGAUCUUC ACAGUUGGAGUUUGA	ID. NO. 280
1410	CUGGAUCUUCACAGUU GGAGUUUGACCCAAA	ID. NO. 281
1416	CUUCACAGUUGGAGUU UGACCCAAAUGCAAA	ID. NO. 282
1417	UUCACAGUUGGAGUUU GACCCAAAUGCAAAG	ID. NO. 283
1448	AAAGUGACACACACUU UGAAGAGUAACAGCU	ID. NO. 284
1449	AAGUGACACACACUUU GAAGAGUAACAGCUG	ID. NO. 285
1457	CACACUUUGAAGAGUA ACAGCUGGCUUAAUU	ID. NO. 286
1468	GAGUAAACAGCUGGCUU AAUUGUUGAAAGAGA	ID. NO. 287
1469	AGUAAACAGCUGGCUUA AUUGUUGAAAGAGAU	ID. NO. 288
1472	AACAGCUGGCUUAAUU GUUGAAAGAGAUUG	ID. NO. 289
1475	AGCUGGCUUAAUUGUU GAAAGAGAUUGUAG	ID. NO. 290
1485	AUUGUUGAAAGAGALIA UGUAGAAGGCACAAU	ID. NO. 291
1489	UUGAAAGAGAUUGUA GAAGGCACAALUUGG	ID. NO. 292
1501	UGUAGAAGGCACAALIA UGGGCACUUUAAUUG	ID. NO. 293
1510	CACAALUUGGGCACUU UAAALUGAAGCUAALIA	ID. NO. 294
1511	ACAALUUGGGCACUUU AAAUGAAGCUAALUAA	ID. NO. 295
1512	CAALUUGGGCACUUUA AALUGAAGCUAALUAAU	ID. NO. 296
1522	ACUUUAAAUGAAGCUA ALUAAUUCUUCACCUA	ID. NO. 297
1525	UUAAAUGAAGCUAALIA AUUCUUCACCUAAGU	ID. NO. 298
1528	AAUGAAGCUAALUAAUU CUUCACCUAAGUCUC	ID. NO. 299
1529	ALUGAAGCUAALUAAUUC UUCACCUAAGUCUCU	ID. NO. 300
1531	GAAGCUAALUAAUUCUU CACCUAAGUCUCUGU	ID. NO. 301
1532	AAGCUAALUAAUUCUUC ACCUAAGUCUCUGUG	ID. NO. 302
1537	AAUAAUUCUUCACCUA AGUCUCUGUGAAUUG	ID. NO. 303
1541	AUUCUUCACCUAAGUC UCUGUGAAUUGAAAU	ID. NO. 304
1543	UCUUCACCUAAGUCUC UGUGAAUUGAAUUGU	ID. NO. 305
1551	UAAGUCUCUGUGAAUU GAAAUUGUUGUUUC	ID. NO. 306
1559	UGUGAAUUGAAUUGUU CGUUUUUCCUGCCU	ID. NO. 307
1560	GUGAAUUGAAUUGUUC GUUUUCCUGCCUG	ID. NO. 308
1563	AAUUGAAAUUGUUGUU UUCUCCUGCCUGGUC	ID. NO. 309
1564	AUUGAAAUUGUUGUUU UCUCUCCUGCCUGGCU	ID. NO. 310
1565	UUGAAAUUGUUGUUUU CUCCUGCCUGGUCUG	ID. NO. 311
1566	UGAAAUUGUUGUUUUC UCCUGCCUGGUCUGU	ID. NO. 312

1568	AA AUGUUUGUUUUCUC CUGCCUGUGCUGUGA	ID. NO. 313
1586	GCCUGUGCUGUGACUC GAGUCACACUCAAGG	ID. NO. 314
1591	UGCUGUGACUUGAGUC ACACUCAAGGGAAU	ID. NO. 315
1597	GACUUGAGUCACACUC AAGGGAACUUGAGOG	ID. NO. 316
1607	ACACUCAAGGGAAUUC GAGCGUGAUCUGUA	ID. NO. 317
1618	AACUUGAGCGUGAUC UGUUUCUUGCCGGUC	ID. NO. 318
1622	UGAGCGUGAUCUGUA UCUUGCCGGUCAUUU	ID. NO. 319
1624	AGCGUGAUCUGUAUC UUGCCGGUCAUUUU	ID. NO. 320
1626	CGUGAUCUGUAUCUU GCGGUGCAUUUUUAU	ID. NO. 321
1633	CUGUAUCUUGCCGGUC AUUUUUAUGUUAUA	ID. NO. 322
1636	UAUCUUGCCGGUCAU UUAUGUUAUUAACAG	ID. NO. 323
1637	AUCUUGCCGGUCAUU UUAUGUUAUUAACAGG	ID. NO. 324
1638	UCUUGCCGGUCAUUU UAUGUUAUUAACAGG	ID. NO. 325
1639	CUUGCCGGUCAUUUU AUGUUAUUAACAGGC	ID. NO. 326
1640	UUGCCGGUCAUUUUA UGUUAUUAACAGGCA	ID. NO. 327
1644	CGGCAUUUUUAUGU AUUAACAGGGCAUUA	ID. NO. 328
1645	GGCAUUUUUAUGUA UUAACAGGGCAUUA	ID. NO. 329
1647	UCAUUUUUAUGUUAU ACAGGGCAUUAUAAU	ID. NO. 330
1648	CAUUUUUAUGUUAUA CAGGGCAUUAUAAUG	ID. NO. 331
1657	GUUAUUAACAGGGCAU CAAUGGGCUGCUGC	ID. NO. 332
1658	UUAUUAACAGGGCAUC AA AUGGGCUGCUGCU	ID. NO. 333
1674	AA AUGGGCUGCUGCUU AGCUUGCACCUUGUC	ID. NO. 334
1675	AAUGGGCUGCUGCUUA GCUUGCACCUUGUCA	ID. NO. 335
1679	GGCUGCUGCUUAGCUU GCACCUUGUCACAU	ID. NO. 336
1686	GCUUAGCUUGCACCUU GUCACAUAGAGUGAU	ID. NO. 337
1689	UAGCUUGCACCUUGUC ACAUAGAGUGAUCUU	ID. NO. 338
1694	UGCACCUUGUCACAU GAGUGAUCUUUCCCA	ID. NO. 339
1702	GUCACAUAGAGUGAUC UUUCCCAAGAGAAGG	ID. NO. 340
1704	CACAUAGAGUGAUCUU UCCCAAGAGAAGGGG	ID. NO. 341
1705	ACAUAGAGUGAUCUUU CCCAAGAGAAGGGGA	ID. NO. 342
1706	CAUAGAGUGAUCUUC CCAAGAGAAGGGGAA	ID. NO. 343
1727	AGAAGGGGAAGCACUC GUGUGCAACAGACAA	ID. NO. 344
1751	CAGACAAGUGACUGUA UCUGUGUAGACUAUU	ID. NO. 345
1753	GACAAGUGACUGUAUC UGUGUAGACUAUUUG	ID. NO. 346
1759	UGACUGUAUCUGUGUA GACUAUUUGCUUAU	ID. NO. 347
1764	GUUUCUGUGUAGACUA UUUGCUUAUUUAUA	ID. NO. 348

1766	AUCUGUGUAGACUAAU UGCUAAUUUAAUAAA	ID. NO. 349
1767	UCUGUGUAGACUAAUU GCUAAUUUAAUAAAG	ID. NO. 350
1771	UGUAGACUAAUUGCUU AUUUAUAAAGACGA	ID. NO. 351
1772	GUAGACUAAUUGCUUA UUAUAUAAAGACGAU	ID. NO. 352
1774	AGACUAAUUGCUAAU UAUAUAAAGACGAUUU	ID. NO. 353
1775	GACUAAUUGCUAAUU AUAUAAAGACGAUUUG	ID. NO. 354
1776	ACUAAUUGCUAAUUA AUAAAGACGAUUUGU	ID. NO. 355
1779	AUUUGCUAAUUAUA AAGACGAUUUGUCAG	ID. NO. 356
1788	UUUAUAUAAAGACGAU UGUCAGUUGUUUU	ID. NO. 357
1789	UUAUAUAAAGACGAUU GUCAGUUGUUUU	ID. NO. 358
1792	AUAUAAAGACGAUUUGC AGUUGUUUU	ID. NO. 359

Table AIV: Human Stromelysin HP Target Sequence

nt. Position	Target Sequence	Seq. ID. NO.
66	CUACU GUU GCUGUGGUGGCAGU	ID. NO. 360
82	UGGCA GUU UGCUCAGOCUAUCCA	ID. NO. 361
192	AAACA GUU UGUUAGGAGAAAGGA	ID. NO. 362
430	AUGCU GUU GAUUCUGCUGUUGAG	ID. NO. 363
442	CUGCU GUU GAGAAAGCUCUGAAA	ID. NO. 364
775	UCACA GAC CUGACUCCGUUCCGC	ID. NO. 365
1360	AUGCU GUU UUUGAAGAAUUGGG	ID. NO. 366
1407	UCACA GUU GGAGUUUGACCCAAA	ID. NO. 367

Table AV: Human HH Ribozyme Sequence

nt. Position.	Ribozyme Sequence	Seq.	ID.
10	GUUGUCUC CUGAAGAGCACGAAAGUGCGAA AUGCCUUG	ID.NO.375	
21	UUAGCUC CUGAUGAGGCCGAAAGGCCGAA AUGUUGU	ID.NO.376	
168	GAGGUCG CUGAUGAGGCCGAAAGGCCGAA AGUAGUU	ID.NO.377	
616	CUCCAUU CUGAUGAGGCCGAAAGGCCGAA AUCCCU	ID.NO.378	
617	UCUCCAU CUGAUGAGGCCGAAAGGCCGAA AAUCCU	ID.NO.379	
633	CAUCAUCA CUGAAGAGCACGAAAGUGCGAA AGUGGGCA	ID.NO.380	
634	UCAUCAUC CUGAAGAGCACGAAAGUGCGAA AAGUGGGC	ID.NO.381	
662	CCUGUUG CUGAUGAGGCCGAAAGGCCGAA AUCCUU	ID.NO.382	
711	ACCCAGG CUGAUGAGGCCGAAAGGCCGAA AGUGGCC	ID.NO.383	
820	GGGACUG CUGAUGAGGCCGAAAGGCCGAA AUGCCAU	ID.NO.384	
883	UCUGGAGG CUGAAGAGCACGAAAGUGCGAA ACAGGUUC	ID.NO.385	
947	CCCUCA CUGAUGAGGCCGAAAGGCCGAA AGUGCUG	ID.NO.386	
996	CCUGAGG CUGAUGAGGCCGAAAGGCCGAA AUUUGCG	ID.NO.387	
1123	UGGCCCA CUGAUGAGGCCGAAAGGCCGAA AAUUGAU	ID.NO.388	
1132	UUUCCUCU CUGAUGAGCACGAAAGUGCGAA AUGGCCCA	ID.NO.389	
1221	CCUUAUCA CUGAAGAGCACGAAAGUGCGAA AAAUGGCU	ID.NO.390	
1266	UCUCCAG CUGAUGAGGCCGAAAGGCCGAA AUUUGUC	ID.NO.391	
1275	UCUCAUCA CUGAAGAGCACGAAAGUGCGAA AUCUCCAG	ID.NO.392	
1334	AUCCUUG CUGAUGAGGCCGAAAGGCCGAA AAAGUCU	ID.NO.393	
1354	CAGCAUC CUGAUGAGGCCGAAAGGCCGAA AUCUUUG	ID.NO.394	
1363	UCUUCAAA CUGAUGAGCACGAAAGUGCGAA ACAGCAUC	ID.NO.395	
1410	AAACUCC CUGAUGAGGCCGAAAGGCCGAA ACUGUGA	ID.NO.396	

Table AVI: Rabbit Stromelysin HH Ribozyme Target Sequence

nt. Position	Target Sequence	nt. Position	Target Sequence
18	CAAGGCAU C AAGACAGC	345	CCUGAUGU U GGUCACUU
29	GACAGCAU A GAGCUGAG	349	AUGUUGGU C ACUUCAGU
39	AGCUGAGU A AAGCCAAU	353	UGGUCACU U CAGUACCU
61	UGAAAACU C UUCAACC	354	GGUCACUU C AGUACCUU
63	AAAACUCU U CCAACCCU	358	ACUUCAGU A CCUUCUUU
64	AAACUCUU C CAACCCUG	362	CAGUACCU U CCCUGGCA
75	ACCCUGCU A CUGCUGUG	363	AGUACCUU C CCUGGCAC
93	GUGGCGCU U UGCUAGC	391	CAAAAACU C ACCUAACT
94	UGGCGCUU U GCUCAGCC	396	ACUCACCU A ACUUCACG
98	GCUUUGCU C AGCCUAUC	400	ACCUAACT U ACAGGAUU
104	CUCAGCCU A UCCACUGG	401	CCUAACTU A CAGGAUUG
106	CAGCCUAU C CACUGGAU	408	UACAGGAU U GUGAAUUA
122	UGGAGCCU C AAGGGAUG	415	UUGUGAAU U ACACACCG
153	AUGGACCU U CUUCAGCA	416	UGUGAAUU A CACACCGG
154	UGGACCUU C UUCAGCAA	427	CACCGGAU C UGCCAAGA
156	GACCUUCU U CAGCAUAU	444	GAUGCUGU U GAUGCUGC
157	ACCUUCUU C AGCAUAU	456	GCUGCCAU U GAGAAAGC
164	UCAGCAAU A UCUGGAAA	466	AGAAAGCU C UGAAGGUC
166	AGCAUAU C UGGAAAAC	474	CUGAAGGU C UGGGAGGA
176	GGAAAACU A CUACAACC	490	AGGUGACU C CACUCACG
179	AAACUACU A CAACCUUG	495	ACUCCACU C ACGUUCUC
186	UACAACCU U GAAAAAGA	500	ACUCACGU U CUCCAGGA
206	GAAACAGU U UGUUAAAA	501	CUCACGUU C UCCAGGAA
207	AAACAGUU U GUUAAAAG	503	CACGUUCU C CAGGAAGU
210	CAGUUUGU U AAAAGAAA	512	CAGGAAGU A UGAAGGAG
211	AGUUUGUU A AAAGAAAG	531	GCUGACAU A AUGAUCUC
226	AGGACAGU A GUCCUGUU	537	AUA AUGAU C UCUUUUGG
229	ACAGUAGU C CUGUUGUU	539	AAUGAUCU C UUUUGGAG
234	AGUCCUGU U GUUAAAAA	541	UGAUCUCU U UGGAGUC
237	CCUGUUGU U AAAAAAAU	542	GAUCUCUU U UGGAGUCC
238	CUGUUGUU A AAAAAAUC	543	AUCUCUUU U GGAGUCCG
246	AAAAAAAU C CAAGAAAU	549	UUUGGAGU C CGAGAACA
263	GCAGAAGU U CCUUGGCU	565	AUGGAGAU U UUAUCCU
264	CAGAAGUU C CUUGGCUU	566	UGGAGAUU U UAUUCCUU
267	AAGUUCU U GGUUGGA	567	GGAGAUUU U AUUCCUUU
272	CCUUGGCU U GGAGGUGA	568	GAGAUUUU A UUCUUUUU
296	GCUGGACU C CAACACCC	570	GAUUUUUU U CCUUUGA
315	GAGGUGAU A CGCAAGCC	571	AUUUUUUU C CUUUUGAU
336	UGUGGCGU U CCUGAUGU	574	UUUUUUUU U UUGAUGGA
337	GUGGCGUU C CUGAUGUU	575	UAUUCCUU U UGAUGGAC

576	AUUCUUU U GAUGGACC	905	UCCAGGAU C UGGGACCC
594	GGAAUGU U UUGGCUCA	918	ACCCAGU C AUGUGUGA
595	GAAUGUU U UGGCUCAU	928	UGUGUGAU C CAGAUCUG
596	AAUGUUU U GGCUCAUG	934	AUCCAGAU C UGUCCUUC
601	UUUUGGCU C AUGCUUUAU	938	AGAUCUGU C CUUCGAUG
607	CUCAUGCU U AUGCACCU	941	UCUGUCCU U CGAUGCAA
608	UCAUGCUU A UGCACCTG	942	CUGUCCUU C GAUGCAAU
627	CCAGGAU U AAUGGAGA	951	GAUGCAAU C AGCACUCU
628	CAGGAUU A AUGGAGAU	958	UCAGCAU C UGAGGGGA
644	UGCCACU U UGAUGAUG	972	GGAGAAU U CUGUUCUU
645	GCCACUU U GAUGAUGA	973	GAGAAAU C UGUUCUUU
673	CAAAGAU A CAACAGGA	977	AAUUCUGU U CUUUAAG
688	GAACCAU U UAUCCUU	978	AUUCUGUU C UUUAAAGA
689	AACCAUU U AUUCCUUG	980	UCUGUUCU U UAAAGACA
690	ACCAUUU A UUCCUUGU	981	CUGUUCUU U AAAGACAG
692	CAUUUAU U CCUUGUUG	982	UGUUCUUU A AAGACAGG
693	AAUUUAU C CUUGUUGC	992	AGACAGGU A UUUCUGGC
696	UUAUUCU U GUUGCUGC	994	ACAGGUU U UCUGGCGC
699	UUCUUGU U GCUGCUC	995	CAGGUUU U CUGGCGCA
706	UUGCUGCU C AUGAGCUU	996	AGGUUUU C UGGCGCAA
714	CAUGAGCU U GGCCACUC	1007	GCGCAAGU C CCUCAGGA
722	UGCCACU C CCUGGGUC	1011	AAGUCCU C AGGAUUCU
730	CCUGGGU C UGUUUCAC	1017	CUCAGGAU U CUCGAACC
734	GGGUCUGU U UCACUCGG	1018	UCAGGAU C UCGAACCU
735	GGUCUGUU U CACUCGGC	1020	AGGAUUCU C GAACCUGA
736	GUCUGUUU C ACUCGGCC	1031	ACCUGAGU U UCAUUUGA
740	GUUUCACU C GGCCAACC	1032	CCUGAGUU U CAUUUGAU
764	GCUGAUGU A CCCAGUCU	1033	CUGAGUUU C AUUUGAUC
771	UACCCAGU C UACAACGC	1036	AGUUUCAU U UGAUCUCU
773	CCCAGUCU A CAACGCCU	1037	GUUUCAUU U GAUCUCUU
782	CAACGCCU U CACAGACC	1041	CAUUUGAU C UCUUCAU
783	AACGCCU C ACAGACCU	1043	UUUGAUCU C UUCAUUCU
800	GGCCCGGU U CCGCCUUU	1045	UGAUCUCU U CAUUCUGG
801	GCCCGGUU C CGCCUUUC	1046	GAUCUCUU C AUUCUGGC
807	UCCCGCCU U UCUCAAGA	1049	CUCUUCAU U CUGGCCAU
808	UCCGCCUU U CUCAAGAU	1050	UCUUCAU C UGGCCAUC
809	CCGCCUUU C UCAAGAU	1058	CUGGCCAU C UCUUCCUU
811	GCCUUUCU C AAGAUGAU	1060	GGCCAUCU C UUCUUUCA
831	GAUGGCAU C CAAUCCCU	1062	CCAUCUCU U CCUUCAGC
836	CAUCCAAU C CCUCUAUG	1063	CAUCUCUU C CUUCAGCA
840	CAAUCCCU C UAUGGACC	1066	CUCUUCU U CAGCAGUG
842	AUCCUCU A UGGACCGG	1067	UCUUCUU C AGCAGUGG
860	CCUUGCCU C UCCUGAUA	1085	UGCUGCAU A UGAAGUUA
862	CUGCCUCU C CUGAUAA	1092	UAUGAAGU U AUUAGCAG
868	CUCCUGAU A ACUCUGGA	1093	AUGAAGUU A UUAGCAGG
872	UGAUAACU C UGGAGUGC	1095	GAAGUUU A AGCAGGGA
883	GAGUGCCU A UGGAACCU	1096	AAGUUUU A GCAGGGAU
894	GAACCGU C CCUCCAGG	1105	GCAGGGAU A CUGUUUUC
898	CUGUCCU C CAGGAUCU	1110	GAUACUGU U UUCAUUUU

1111	AUACUGUU U UCAUUUUU	1374	GAUGCUGU U UUUGAAGC
1112	UACUGUUU U CAUUUUUA	1375	AUGCUGUU U UUGAAGCA
1113	ACUGUUUU C AUUUUUA	1376	UGCUGUUU U UGAAGCAU
1116	GUUUUCAU U UUUAAAGG	1377	GCUGUUUU U GAAGCAUU
1117	UUUUCAUU U UUAAGGA	1385	UGAAGCAU U UGGGUUUU
1118	UUUCAUUU U UAAAGGAA	1386	GAAGCAUU U GGGUUUUU
1119	UUCAUUUU U AAAGGAAC	1391	AUUUGGGU U UUUCUAUU
1120	UCAUUUUU A AAGGAACU	1392	UUUGGGUU U UUCUAUUU
1129	AAGGAACU C AGUUCUGG	1393	UUGGGUUU U UCUAUUUC
1133	AACUCAGU U CUGGGCCA	1394	UGGGUUUU U CUAUUUCU
1134	ACUCAGUU C UGGGCCAU	1395	GGGUUUUU C UAUUUUCU
1143	UGGGCCAU U AGAGGAAA	1397	GUUUUUUU A UUUCUUA
1144	GGGCCAUU A GAGGAAAU	1399	UUUUCUAU U UCUUCAGU
1158	AAUGAGGU A CAAGCUGG	1400	UUUCUAUU U CUUCAGUG
1168	AAGCUGGU U ACCCAAGA	1401	UUCUAUUU C UUCAGUGG
1169	AGCUGGUU A CCCAAGAA	1403	CUAUUUUU U CAGUGGAU
1182	AGAAGCAU C CACACCCU	1404	UAUUUCUU C AGUGGAUC
1195	CCUGGGU U UCCCUUCA	1412	CAGUGGAU C UUCACAGU
1196	CCUGGGUU U CCCUCAA	1414	GUGGAUCU U CACAGUCG
1197	CUGGGUUU C CCUUCAAC	1415	UGGAUCUU C ACAGUCGG
1201	GUUUCCCU U CAACCAUA	1421	UUCACAGU C GGAGUUUG
1202	UUUCCCUU C AACCAUAA	1427	GUCGGAGU U UGACCCAA
1209	UCAACCAU A AGAAAAAU	1428	UCGGAGUU U GACCCAAA
1218	AGAAAAAU U GAUGCUGC	1458	ACACAUGU U UUGAAGAG
1230	GCUGCCAU U UCUGAUAA	1459	CACAUGUU U UGAAGAGC
1231	CUGCCAUU U CUGAUAA	1460	ACAUGUUU U GAAGAGCA
1232	UGCCAUUU C UGAUAAGG	1478	CAGCUGGU U UCAGUGUU
1237	UUUCUGAU A AGGAAAGG	1479	AGCUGGUU U CAGUGUUA
1256	GAAAACAU A CUUCUUUG	1480	GCUGGUUU C AGUGUUAG
1259	AACAUACU U CUUUGUGG	1486	UUCAGUGU U AGGAGGGG
1260	ACAUACUU C UUUGUGGA	1487	UCAGUGUU A GGAGGGGU
1262	AUACUUUU U UGUGGAAG	1498	AGGGGUGU A UAGAAGGC
1263	UACUUCUU U GUGGAAGA	1500	GGGUGUUA A GAAGGCAC
1277	AGACAAAU A CUGGAGGU	1519	AUGAAUGU U UUAUAUGA
1286	CUGGAGGU U UGAUGAGA	1520	UGAAUGUU U UAAAUUGA
1287	UGGAGGUU U GAUGAGAA	1521	GAAUGUUU U AAUUGAAC
1304	GAGACAGU C CCUGGAGC	1522	AAUGUUUU A AAUGAACC
1319	GCCAGGCU U UCCAGAGC	1532	AUGAACCU A AUUGUUCA
1320	CCAGGCUU U CCCAGACA	1535	AACCUAAU U GUUCAACA
1321	CAGGCUUU C CCAGACAU	1538	CUAAUUGU U CAACACUU
1330	CCAGACAU A UAGCAGAA	1539	UAAUUGUU C AACACUUA
1332	AGACAUUU A GCAGAAGA	1546	UCAACACU U AGGACUUU
1343	AGAAGACU U UCCAGGAA	1547	CAACACUU A GGACUUUG
1344	GAAGACUU U CCAGGAAU	1553	UUAGGACU U UGUGAGUU
1345	AAGACUUU C CAGGAAUU	1554	UAGGACUU U GUGAGUUG
1353	CCAGGAAU U AAUCCAAA	1561	UUGUGAGU U GAAGUGGC
1354	CAGGAAUU A AUCCAAAG	1571	AAGUGGCU C AUUUUCUC
1357	GAAUUAAU C CAAAGAUC	1574	UGGCUCAU U UUCUCCUG
1365	CCAAAGAU C GAUGCUGU	1575	GGCUCAUU U UCUCUCCG

1576 GCUCAUUU U CUCCUGCA
1577 CUCAUUUU C UCCUGCAU
1579 CAUUUUCU C CUGCAUAU
1586 UCCUGCAU A UGCUGUGA
1602 AUGGGAU C UCGAGCAU
1604 GGGAAUCU C GAGCAUGA
1620 AACUGUGU A UCUAACUG
1622 CUGUGUAU C UAACUGGA
1624 GUGUAUCU A ACUGGACU
1633 ACUGGACU U UGCACAUC
1634 CUGGACUU U GCACAUCG
1641 UUGCACAU C GUUACGGG
1644 CACAUCGU U ACGGGUGU
1645 ACAUCGUU A CGGGUGUU
1653 ACGGGUGU U CAAACAGG
1654 CGGGUGUU C AAACAGGC
1670 CUGCUGCU U AGCUUGCA
1671 UGCUGCUU A GCUUGCAC
1675 GCUUAGCU U GCACUUGA
1681 CUUGCACU U GAUCACAU
1685 CACUUGAU C ACAUGGAA
1701 AGGGAGCU U CCACGAGA
1702 GGGAGCUU C CACGAGAC
1720 GGGGAAGU A CUCAUGUG
1723 GAAGUACU C AUGUGUGA
1744 CGAGUGAU U GUGUCUAU
1749 GAUUGUGU C UAUGUGGA
1751 UUGUGUCU A UGUGGAUU
1759 AUGUGGAU U AUUUGCCC
1760 UGUGGAUU A UUUGCCCA
1762 UGGAUUAU U UGCCCCAU
1763 GGAUUAUU U GCCCAUUA
1770 UUGCCCAU U AUUUAUA
1771 UGCCCAUU A UUUAUAUA
1773 CCCAUUAU U UAAUAAAG
1774 CCAUUAUU U AAUAAAGA
1775 CAUUAUUU A AUAAAGAG
1778 UAUUUAUU A AAGAGGAU
1787 AAGAGGAU U UGUCAAUU

Table AVII: Rabbit Stromelysin HH Ribozyme Sequence

nt. Position	Ribozyme Sequence
18	GCUGUCUU CUGAUGAGGCCGAAAGGCCGAA AUGCCUUG
29	CUCAGCUC CUGAUGAGGCCGAAAGGCCGAA AUGCUGUC
39	AUUGGCUU CUGAUGAGGCCGAAAGGCCGAA ACUCAGCU
61	GGUUGGAA CUGAUGAGGCCGAAAGGCCGAA AGUUUUA
63	AGGUUGG CUGAUGAGGCCGAAAGGCCGAA AGAGUUUU
64	CAGGUUG CUGAUGAGGCCGAAAGGCCGAA AAGAGUUU
75	CACAGCAG CUGAUGAGGCCGAAAGGCCGAA AGCAGGGU
93	GCUGAGCA CUGAUGAGGCCGAAAGGCCGAA AGCGCCAC
94	GGCUGAGC CUGAUGAGGCCGAAAGGCCGAA AAGCGCCA
98	GAUAGGCU CUGAUGAGGCCGAAAGGCCGAA AGCAAAGC
104	CCAGUGGA CUGAUGAGGCCGAAAGGCCGAA AGGCUGAG
106	AUCCAGUG CUGAUGAGGCCGAAAGGCCGAA AUAGGCUG
122	CAUCCCUU CUGAUGAGGCCGAAAGGCCGAA AGGCUCCA
153	UGCUGAAG CUGAUGAGGCCGAAAGGCCGAA AGGUCCAU
154	UUGCUGAA CUGAUGAGGCCGAAAGGCCGAA AAGGUCCA
156	UAUUGCUG CUGAUGAGGCCGAAAGGCCGAA AGAAGGUC
157	AUAUUGCU CUGAUGAGGCCGAAAGGCCGAA AGAAGGUA
164	UUUCCAGA CUGAUGAGGCCGAAAGGCCGAA AUUGCUGA
166	GUUUUCCA CUGAUGAGGCCGAAAGGCCGAA AUUUGCUU
176	GGUUGUAG CUGAUGAGGCCGAAAGGCCGAA AGUUUUCC
179	CAAGGUUG CUGAUGAGGCCGAAAGGCCGAA AGUAGUUU
186	UCUUUUUC CUGAUGAGGCCGAAAGGCCGAA AGGUUGUA
206	UUUUUACA CUGAUGAGGCCGAAAGGCCGAA ACUGUUUC
207	CUUUUAA CUGAUGAGGCCGAAAGGCCGAA AACUGUUU
210	UUUCUUUU CUGAUGAGGCCGAAAGGCCGAA ACAAACUG
211	CUUUCUUU CUGAUGAGGCCGAAAGGCCGAA AACAAACU
226	AACAGGAC CUGAUGAGGCCGAAAGGCCGAA ACUGUCCU
229	AACAACAG CUGAUGAGGCCGAAAGGCCGAA ACUACUGU
234	UUUUUAA CUGAUGAGGCCGAAAGGCCGAA ACAGGACU
237	AUUUUUUU CUGAUGAGGCCGAAAGGCCGAA ACAACAGG
238	GAUUUUUU CUGAUGAGGCCGAAAGGCCGAA AACAACAG
246	AUUUCUUG CUGAUGAGGCCGAAAGGCCGAA AUUUUUUU
263	AGCCAAGG CUGAUGAGGCCGAAAGGCCGAA ACUUCUGC
264	AAGCCAAG CUGAUGAGGCCGAAAGGCCGAA AACUUCUG
267	UCCAAGCC CUGAUGAGGCCGAAAGGCCGAA AGGAACUU
272	UCACCUCC CUGAUGAGGCCGAAAGGCCGAA AGCCAAGG
296	GGGUGUUG CUGAUGAGGCCGAAAGGCCGAA AGUCCAGC
315	GGCUUGCG CUGAUGAGGCCGAAAGGCCGAA AUCACCUC
336	ACAUCAGG CUGAUGAGGCCGAAAGGCCGAA ACGCCACA
337	AACAUCAG CUGAUGAGGCCGAAAGGCCGAA AACGCCAC
345	AAGUGACC CUGAUGAGGCCGAAAGGCCGAA ACAUCAGG

349	ACUGAAGU	CUGAUGAGGCCGAAAGGCCGAA	ACCAACAU
353	AGGUACUG	CUGAUGAGGCCGAAAGGCCGAA	AGUGACCA
354	AAGGUACU	CUGAUGAGGCCGAAAGGCCGAA	AAGUGACC
358	AGGGAAGG	CUGAUGAGGCCGAAAGGCCGAA	ACUGAAGU
362	UGCCAGGG	CUGAUGAGGCCGAAAGGCCGAA	AGGUACUG
363	GUGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGUACU
391	AGUUAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGUUUUUG
396	CUGUAAGU	CUGAUGAGGCCGAAAGGCCGAA	AGGUGAGU
400	AAUCCUGU	CUGAUGAGGCCGAAAGGCCGAA	AGUUAGGU
401	CAAUCCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGUUAGG
408	UAAUUCAC	CUGAUGAGGCCGAAAGGCCGAA	AUCCUGUA
415	CGGUGUGU	CUGAUGAGGCCGAAAGGCCGAA	AUUCACAA
416	CCGGUGUG	CUGAUGAGGCCGAAAGGCCGAA	AAUUCACA
427	UCUUGGCA	CUGAUGAGGCCGAAAGGCCGAA	AUCCGGUG
444	GCAGCAUC	CUGAUGAGGCCGAAAGGCCGAA	ACAGCAUC
456	GCUUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AUGGCAGC
466	GACCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUUUCU
474	UCCUCCCA	CUGAUGAGGCCGAAAGGCCGAA	ACCUUCAG
490	CGUGAGUG	CUGAUGAGGCCGAAAGGCCGAA	AGUCACCU
495	GAGAACGU	CUGAUGAGGCCGAAAGGCCGAA	AGUGGAGU
500	UCCUGGAG	CUGAUGAGGCCGAAAGGCCGAA	ACGUGAGU
501	UUCUUGGA	CUGAUGAGGCCGAAAGGCCGAA	AACGUGAG
503	ACUUCCTUG	CUGAUGAGGCCGAAAGGCCGAA	AGAACGUG
512	CUCCUUCA	CUGAUGAGGCCGAAAGGCCGAA	ACUUCCTUG
531	GAGAUCAU	CUGAUGAGGCCGAAAGGCCGAA	AUGUCAGC
537	CCAAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AUCAUUAU
539	CUCCAAAA	CUGAUGAGGCCGAAAGGCCGAA	AGAUCAUU
541	GACUCCAA	CUGAUGAGGCCGAAAGGCCGAA	AGAGAUCA
542	GGACUCCA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAUC
543	CGGACUCC	CUGAUGAGGCCGAAAGGCCGAA	AAAGAGAU
549	UGUUCUCG	CUGAUGAGGCCGAAAGGCCGAA	ACUCCAAA
565	AGGAAUAA	CUGAUGAGGCCGAAAGGCCGAA	AUCUCCAU
566	AAGGAUUA	CUGAUGAGGCCGAAAGGCCGAA	AAUCUCCA
567	AAAGGAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAUCUCC
568	AAAAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AAAAUCUC
570	UCAAAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAAAAUC
571	AUCAAAG	CUGAUGAGGCCGAAAGGCCGAA	AAUAAAAU
574	UCCAUCAA	CUGAUGAGGCCGAAAGGCCGAA	AGGAAUAA
575	GUCCAUCA	CUGAUGAGGCCGAAAGGCCGAA	AAGGAAUA
576	GGUCCAUC	CUGAUGAGGCCGAAAGGCCGAA	AAAGGAAU
594	UGAGCCAA	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUCC
595	AUGAGCCA	CUGAUGAGGCCGAAAGGCCGAA	AACAUUUC
596	CAUGAGCC	CUGAUGAGGCCGAAAGGCCGAA	AAACAUUU
601	AUAAGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCCAAAA
607	AGGUGCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAUGAG
608	CAGGUGCA	CUGAUGAGGCCGAAAGGCCGAA	AAGCAUGA
627	UCUCCAUU	CUGAUGAGGCCGAAAGGCCGAA	AUUCUUGG
628	AUCUCCAU	CUGAUGAGGCCGAAAGGCCGAA	AAUUCCTUG
644	CAUCAUCA	CUGAUGAGGCCGAAAGGCCGAA	AGUGGGCA

645 UCAUCAUC CUGAUGAGGCCGAAAGGCCGAA AAGUGGGC
673 UCCUGUUG CUGAUGAGGCCGAAAGGCCGAA AUCCUUUG
688 AAGGAUA CUGAUGAGGCCGAAAGGCCGAA AUUGGUUC
689 CAAGGAU CUGAUGAGGCCGAAAGGCCGAA AAUUGGUU
690 ACAAGGAA CUGAUGAGGCCGAAAGGCCGAA AAAUUGGU
692 CAACAAG CUGAUGAGGCCGAAAGGCCGAA AUAAAUUG
693 GCAACAAG CUGAUGAGGCCGAAAGGCCGAA AAUAAUUG
696 GCAGCAAC CUGAUGAGGCCGAAAGGCCGAA AGGAAUAA
699 UGAGCAGC CUGAUGAGGCCGAAAGGCCGAA ACAAGGAA
706 AAGCUCAU CUGAUGAGGCCGAAAGGCCGAA AGCAGCAA
714 GAGUGGCC CUGAUGAGGCCGAAAGGCCGAA AGCUCAUG
722 GACCCAGG CUGAUGAGGCCGAAAGGCCGAA AGUGGCCA
730 GUGAAACA CUGAUGAGGCCGAAAGGCCGAA ACCCAGGG
734 CCGAGUGA CUGAUGAGGCCGAAAGGCCGAA ACAGACCC
735 GCGAGUG CUGAUGAGGCCGAAAGGCCGAA AACAGACC
736 GGCCGAGU CUGAUGAGGCCGAAAGGCCGAA AAACAGAC
740 GGUUGGCC CUGAUGAGGCCGAAAGGCCGAA AGUGAAAC
764 AGACUGGG CUGAUGAGGCCGAAAGGCCGAA ACAUCAGC
771 GCGUUGUA CUGAUGAGGCCGAAAGGCCGAA ACUGGGUA
773 AGGCGUUG CUGAUGAGGCCGAAAGGCCGAA AGACUGGG
782 GGUCUGUG CUGAUGAGGCCGAAAGGCCGAA AGGCGUUG
783 AGGUCUGU CUGAUGAGGCCGAAAGGCCGAA AAGGCGUU
800 AAAGGCGG CUGAUGAGGCCGAAAGGCCGAA ACCGGGCC
801 GAAAGGCG CUGAUGAGGCCGAAAGGCCGAA AACCGGGC
807 UCUUGAGA CUGAUGAGGCCGAAAGGCCGAA AGGCGGAA
808 AUCUUGAG CUGAUGAGGCCGAAAGGCCGAA AAGGCGGA
809 CAUCUUGA CUGAUGAGGCCGAAAGGCCGAA AAAGGCGG
811 AUCAUCUU CUGAUGAGGCCGAAAGGCCGAA AGAAAGGC
831 AGGGAUUG CUGAUGAGGCCGAAAGGCCGAA AUGCCAUC
836 CAUAGAGG CUGAUGAGGCCGAAAGGCCGAA AUUGGAUG
840 GGUCCAUA CUGAUGAGGCCGAAAGGCCGAA AGGGAUUG
842 CCGGUCCA CUGAUGAGGCCGAAAGGCCGAA AGAGGGAU
860 UAUCAGGA CUGAUGAGGCCGAAAGGCCGAA AGGCAGGG
862 GUUAUCAG CUGAUGAGGCCGAAAGGCCGAA AGAGGCAG
868 UCCAGAGU CUGAUGAGGCCGAAAGGCCGAA AUCAGGAG
872 GCACUCCA CUGAUGAGGCCGAAAGGCCGAA AGUUAUCA
883 AGGUUCCA CUGAUGAGGCCGAAAGGCCGAA AGGCACUC
894 CCUGGAGG CUGAUGAGGCCGAAAGGCCGAA ACAGGUUC
898 AGAUCCUG CUGAUGAGGCCGAAAGGCCGAA AGGGACAG
905 GGUUCCA CUGAUGAGGCCGAAAGGCCGAA AUCCUGGA
918 UCACACAU CUGAUGAGGCCGAAAGGCCGAA ACUGGGGU
928 CAGAUUCG CUGAUGAGGCCGAAAGGCCGAA AUCACACA
934 GAAGGACA CUGAUGAGGCCGAAAGGCCGAA AUCUGGAU
938 CAUCGAAG CUGAUGAGGCCGAAAGGCCGAA ACAGAUUC
941 UUGCAUCG CUGAUGAGGCCGAAAGGCCGAA AGGACAGA
942 AUUGCAUC CUGAUGAGGCCGAAAGGCCGAA AAGGACAG
951 AGAGUGCU CUGAUGAGGCCGAAAGGCCGAA AUUGCAUC
958 UCCCTUCA CUGAUGAGGCCGAAAGGCCGAA AGUGCUGA
972 AAGAACAG CUGAUGAGGCCGAAAGGCCGAA AUUUCUCC

973 AAAGAACA CUGAUGAGGCCGAAAGGCCGAA AAUUUCUC
977 CUUUAAAG CUGAUGAGGCCGAAAGGCCGAA ACAGAAUU
978 UCUUUAAA CUGAUGAGGCCGAAAGGCCGAA AACAGAAU
980 UGUUUUA CUGAUGAGGCCGAAAGGCCGAA AGAACAGA
981 CUGUCUU CUGAUGAGGCCGAAAGGCCGAA AAGAACAG
982 CCUGUCU CUGAUGAGGCCGAAAGGCCGAA AAAGAACA
992 GCCAGAAA CUGAUGAGGCCGAAAGGCCGAA ACCUGUCU
994 GCGCCAGA CUGAUGAGGCCGAAAGGCCGAA AUACCUGU
995 UGCGCCAG CUGAUGAGGCCGAAAGGCCGAA AAUACCUG
996 UUGCGCCA CUGAUGAGGCCGAAAGGCCGAA AAAUACCU
1007 UCCUGAGG CUGAUGAGGCCGAAAGGCCGAA ACUUGCGC
1011 AGAAUCCU CUGAUGAGGCCGAAAGGCCGAA AGGGACUU
1017 GGUUCGAG CUGAUGAGGCCGAAAGGCCGAA AUCCUGAG
1018 AGGUUCGA CUGAUGAGGCCGAAAGGCCGAA AAUCCUGA
1020 UCAGGUUC CUGAUGAGGCCGAAAGGCCGAA AGAAUCCU
1031 UCAAAUGA CUGAUGAGGCCGAAAGGCCGAA ACUCAGGU
1032 AUCAAAUG CUGAUGAGGCCGAAAGGCCGAA AACUCAGG
1033 GAUCAAUU CUGAUGAGGCCGAAAGGCCGAA AAACUCAG
1036 AGAGAUCA CUGAUGAGGCCGAAAGGCCGAA AUGAAACU
1037 AAGAGAU CUGAUGAGGCCGAAAGGCCGAA AAUGAAAC
1041 AAUGAAGA CUGAUGAGGCCGAAAGGCCGAA AUCAAUUG
1043 AGAAUGAA CUGAUGAGGCCGAAAGGCCGAA AGAUCAAA
1045 CCAGAAUG CUGAUGAGGCCGAAAGGCCGAA AGAGAUCA
1046 GCCAGAAU CUGAUGAGGCCGAAAGGCCGAA AAGAGAU C
1049 AUGGCCAG CUGAUGAGGCCGAAAGGCCGAA AUGAAGAG
1050 GAUGGCCA CUGAUGAGGCCGAAAGGCCGAA AAUGAAGA
1058 AAGGAAGA CUGAUGAGGCCGAAAGGCCGAA AUGGCCAG
1060 UGAAGGAA CUGAUGAGGCCGAAAGGCCGAA AGAUGGCC
1062 GCUGAAGG CUGAUGAGGCCGAAAGGCCGAA AGAGAUUG
1063 UGCUGAAG CUGAUGAGGCCGAAAGGCCGAA AAGAGAU G
1066 CACUGCUG CUGAUGAGGCCGAAAGGCCGAA AGGAAGAG
1067 CCACUGCU CUGAUGAGGCCGAAAGGCCGAA AAGGAAGA
1085 UAACUUCA CUGAUGAGGCCGAAAGGCCGAA AUGCAGCA
1092 CUGCUAAU CUGAUGAGGCCGAAAGGCCGAA ACTUCAUA
1093 CCUGCUAA CUGAUGAGGCCGAAAGGCCGAA AACUUCAU
1095 UCCCUGCU CUGAUGAGGCCGAAAGGCCGAA AUAACTUC
1096 AUCCCUGC CUGAUGAGGCCGAAAGGCCGAA AAUAACUU
1105 GAAAACAG CUGAUGAGGCCGAAAGGCCGAA AUCCCUGC
1110 AAAAUGAA CUGAUGAGGCCGAAAGGCCGAA ACAGUAUC
1111 AAAAAUGA CUGAUGAGGCCGAAAGGCCGAA AACAGUAU
1112 UAAAAAUG CUGAUGAGGCCGAAAGGCCGAA AAACAGUA
1113 UUA AAAAU CUGAUGAGGCCGAAAGGCCGAA AAAACAGU
1116 CCUUUAAA CUGAUGAGGCCGAAAGGCCGAA AUGAAAAC
1117 UCCUUUAA CUGAUGAGGCCGAAAGGCCGAA AAUGAAAA
1118 UUCCUUUA CUGAUGAGGCCGAAAGGCCGAA AAAUGAAA
1119 GUUCCUUU CUGAUGAGGCCGAAAGGCCGAA AAAAUGAA
1120 AGUCCUU CUGAUGAGGCCGAAAGGCCGAA AAAAAUGA
1129 CCAGAACTU CUGAUGAGGCCGAAAGGCCGAA AGUUCCTU
1133 UGGCCAG CUGAUGAGGCCGAAAGGCCGAA ACUGAGTU

1134 AUGGCCCA CUGAUGAGGCCGAAAGGCCGAA AACUGAGU
1143 UUUCCUCU CUGAUGAGGCCGAAAGGCCGAA AUGGCCCA
1144 AUUUCCUC CUGAUGAGGCCGAAAGGCCGAA AAUGGCCC
1158 CCAGCUUG CUGAUGAGGCCGAAAGGCCGAA ACCUCAUU
1168 UCUUGGGU CUGAUGAGGCCGAAAGGCCGAA ACCAGCUU
1169 UUCUUGGG CUGAUGAGGCCGAAAGGCCGAA AACCAGCU
1182 AGGGUGUG CUGAUGAGGCCGAAAGGCCGAA AUGCUUCU
1195 UGAAGGGA CUGAUGAGGCCGAAAGGCCGAA ACCCAGGG
1196 UUGAAGGG CUGAUGAGGCCGAAAGGCCGAA AACCAGG
1197 GUUGAAGG CUGAUGAGGCCGAAAGGCCGAA AAACCCAG
1201 UAUGGUUG CUGAUGAGGCCGAAAGGCCGAA AGGGAAAC
1202 UUAUGGUU CUGAUGAGGCCGAAAGGCCGAA AAGGAAA
1209 AUUUUUCU CUGAUGAGGCCGAAAGGCCGAA AUGGUUGA
1218 GCAGCAUC CUGAUGAGGCCGAAAGGCCGAA AUUUUUCU
1230 UUAUCAGA CUGAUGAGGCCGAAAGGCCGAA AUGGCAGC
1231 CUUAUCAG CUGAUGAGGCCGAAAGGCCGAA AAUGGCAG
1232 CCUAUCA CUGAUGAGGCCGAAAGGCCGAA AAAUGGCA
1237 CCUUUCCU CUGAUGAGGCCGAAAGGCCGAA AUCAGAAA
1256 CAAAGAAG CUGAUGAGGCCGAAAGGCCGAA AUGUUUUC
1259 CCACAAAG CUGAUGAGGCCGAAAGGCCGAA AGUAUGUU
1260 UCCACAAA CUGAUGAGGCCGAAAGGCCGAA AAGUAUGU
1262 CUUCCACA CUGAUGAGGCCGAAAGGCCGAA AGAAGUAU
1263 UCUUCCAC CUGAUGAGGCCGAAAGGCCGAA AAGAAGUA
1277 ACCUCCAG CUGAUGAGGCCGAAAGGCCGAA AUUUGUCU
1286 UCUCAUCA CUGAUGAGGCCGAAAGGCCGAA ACCUCCAG
1287 UUCUCAUC CUGAUGAGGCCGAAAGGCCGAA AACCUCCA
1304 GCUCCAGG CUGAUGAGGCCGAAAGGCCGAA ACUGUCUC
1319 GUCUGGGA CUGAUGAGGCCGAAAGGCCGAA AGCCUGGC
1320 UGUCUGGG CUGAUGAGGCCGAAAGGCCGAA AAGCCUGG
1321 AUGUCUGG CUGAUGAGGCCGAAAGGCCGAA AAAGCCUG
1330 UUCUGCUA CUGAUGAGGCCGAAAGGCCGAA AUGUCUGG
1332 UCUUCUGC CUGAUGAGGCCGAAAGGCCGAA AUAUGUCU
1343 UUCCUGGA CUGAUGAGGCCGAAAGGCCGAA AGUCUUCU
1344 AUUCCUGG CUGAUGAGGCCGAAAGGCCGAA AAGUCUUC
1345 AAUUCUG CUGAUGAGGCCGAAAGGCCGAA AAAGUCUU
1353 UUUGGAUU CUGAUGAGGCCGAAAGGCCGAA AUUCCUGG
1354 CUUUGGAU CUGAUGAGGCCGAAAGGCCGAA AAUUCUG
1357 GAUCUUUG CUGAUGAGGCCGAAAGGCCGAA AUUAAUUC
1365 ACAGCAUC CUGAUGAGGCCGAAAGGCCGAA AUCUUUGG
1374 GCUUCAA CUGAUGAGGCCGAAAGGCCGAA ACAGCAUC
1375 UGCUCAA CUGAUGAGGCCGAAAGGCCGAA AACAGCAU
1376 AUGCUUCA CUGAUGAGGCCGAAAGGCCGAA AAACAGCA
1377 AAUGCUUC CUGAUGAGGCCGAAAGGCCGAA AAAACAGC
1385 AAAACCCA CUGAUGAGGCCGAAAGGCCGAA AUGCUUCA
1386 AAAAACCC CUGAUGAGGCCGAAAGGCCGAA AAUGCUUC
1391 AAUAGAAA CUGAUGAGGCCGAAAGGCCGAA ACCCAAU
1392 AAUAGAA CUGAUGAGGCCGAAAGGCCGAA AACCCAA
1393 GAAAUAGA CUGAUGAGGCCGAAAGGCCGAA AAACCCAA
1394 AGAAAUAG CUGAUGAGGCCGAAAGGCCGAA AAAACCCA

1395 AAGAAUA CUGAUGAGGCCGAAAGGCCGAA AAAAACC
1397 UGAAGAA CUGAUGAGGCCGAAAGGCCGAA AGAAAAAC
1399 ACUGAAGA CUGAUGAGGCCGAAAGGCCGAA AUAGAAAA
1400 CACUGAAG CUGAUGAGGCCGAAAGGCCGAA AAUAGAAA
1401 CCACUGAA CUGAUGAGGCCGAAAGGCCGAA AAUAGAA
1403 AUCCACUG CUGAUGAGGCCGAAAGGCCGAA AGAAAUAG
1404 GAUCCACU CUGAUGAGGCCGAAAGGCCGAA AAGAAUA
1412 ACUGUGAA CUGAUGAGGCCGAAAGGCCGAA AUCCACUG
1414 CGACUGUG CUGAUGAGGCCGAAAGGCCGAA AGAUCCAC
1415 CCGACUGU CUGAUGAGGCCGAAAGGCCGAA AAGAUCCA
1421 CAAACUCC CUGAUGAGGCCGAAAGGCCGAA ACUGUGAA
1427 UUGGGUCA CUGAUGAGGCCGAAAGGCCGAA ACUCCGAC
1428 UUUGGGUC CUGAUGAGGCCGAAAGGCCGAA AACUCCGA
1458 CUCUUCAA CUGAUGAGGCCGAAAGGCCGAA ACAUGUGU
1459 GCUUCUA CUGAUGAGGCCGAAAGGCCGAA AACAUUG
1460 UGCUUCU CUGAUGAGGCCGAAAGGCCGAA AAACAUGU
1478 AACACUGA CUGAUGAGGCCGAAAGGCCGAA ACCAGCUG
1479 UAACACUG CUGAUGAGGCCGAAAGGCCGAA AACCAGCU
1480 CUAACACU CUGAUGAGGCCGAAAGGCCGAA AAACCAGC
1486 CCCCUCU CUGAUGAGGCCGAAAGGCCGAA ACACUGAA
1487 ACCCCUCC CUGAUGAGGCCGAAAGGCCGAA AACACUGA
1498 GCCUUCUA CUGAUGAGGCCGAAAGGCCGAA ACACCCCU
1500 GUGCCUUC CUGAUGAGGCCGAAAGGCCGAA AUACACCC
1519 UCAUUUA CUGAUGAGGCCGAAAGGCCGAA ACAUUCAU
1520 UUCAUUUA CUGAUGAGGCCGAAAGGCCGAA AACAUUCA
1521 GUUCAUU CUGAUGAGGCCGAAAGGCCGAA AAACAUUC
1522 GGUUCAU CUGAUGAGGCCGAAAGGCCGAA AAAACAUU
1532 UGAACAAU CUGAUGAGGCCGAAAGGCCGAA AGGUUCAU
1535 UGUUGAAC CUGAUGAGGCCGAAAGGCCGAA AUUAGGUU
1538 AAGUGUUG CUGAUGAGGCCGAAAGGCCGAA ACAAUUAG
1539 UAAGUGUU CUGAUGAGGCCGAAAGGCCGAA AACAAUUA
1546 AAAGUCCU CUGAUGAGGCCGAAAGGCCGAA AGUGUUGA
1547 CAAAGUCC CUGAUGAGGCCGAAAGGCCGAA AAGUGUUG
1553 AACUCACA CUGAUGAGGCCGAAAGGCCGAA AGUCCUAA
1554 CAACUCAC CUGAUGAGGCCGAAAGGCCGAA AAGUCCUA
1561 GCCACUUC CUGAUGAGGCCGAAAGGCCGAA ACUCACAA
1571 GAGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCCACUU
1574 CAGGAGAA CUGAUGAGGCCGAAAGGCCGAA AUGAGCCA
1575 GCAGGAGA CUGAUGAGGCCGAAAGGCCGAA AAUGAGCC
1576 UGCAGGAG CUGAUGAGGCCGAAAGGCCGAA AAAUGAGC
1577 AUGCAGGA CUGAUGAGGCCGAAAGGCCGAA AAAAUGAG
1579 AUAUGCAG CUGAUGAGGCCGAAAGGCCGAA AGAAAUG
1586 UCACAGCA CUGAUGAGGCCGAAAGGCCGAA AUGCAGGA
1602 AUGCUCGA CUGAUGAGGCCGAAAGGCCGAA AUUCCCAU
1604 UCAUGCUC CUGAUGAGGCCGAAAGGCCGAA AGAUUCCC
1620 CAGUUAGA CUGAUGAGGCCGAAAGGCCGAA ACACAGUU
1622 UCCAGUUA CUGAUGAGGCCGAAAGGCCGAA AUACACAG
1624 AGUCCAGU CUGAUGAGGCCGAAAGGCCGAA AGAUACAC
1633 GAUGUGCA CUGAUGAGGCCGAAAGGCCGAA AGUCCAGU

1634 CGAUGUGC CUGAUGAGGCCGAAAGGCCGAA AAGUCCAG
1641 CCCGUAAC CUGAUGAGGCCGAAAGGCCGAA AUGUGCAA
1644 ACACCCGU CUGAUGAGGCCGAAAGGCCGAA ACGAUGUG
1645 AACACCCG CUGAUGAGGCCGAAAGGCCGAA AACGAUGU
1653 CCUGUUG CUGAUGAGGCCGAAAGGCCGAA ACACCCGU
1654 GCCUGUU CUGAUGAGGCCGAAAGGCCGAA AACACCCG
1670 UGCAAGCU CUGAUGAGGCCGAAAGGCCGAA AGCAGCAG
1671 GUGCAAGC CUGAUGAGGCCGAAAGGCCGAA AAGCAGCA
1675 UCAAGUGC CUGAUGAGGCCGAAAGGCCGAA AGCUAAGC
1681 AUGUGAUC CUGAUGAGGCCGAAAGGCCGAA AGUGCAAG
1685 UUCCAUGU CUGAUGAGGCCGAAAGGCCGAA AUCAAGUG
1701 UCUCGUG CUGAUGAGGCCGAAAGGCCGAA AGCUCUCCU
1702 GUCUCGUG CUGAUGAGGCCGAAAGGCCGAA AAGCUCUCC
1720 CACAUGAG CUGAUGAGGCCGAAAGGCCGAA ACUUCUCCC
1723 UCACACAU CUGAUGAGGCCGAAAGGCCGAA AGUACUUC
1744 AUAGACAC CUGAUGAGGCCGAAAGGCCGAA AUCACUUG
1749 UCCACAU CUGAUGAGGCCGAAAGGCCGAA ACACAAUC
1751 AAUCCACA CUGAUGAGGCCGAAAGGCCGAA AGACACAA
1759 GGGCAAAU CUGAUGAGGCCGAAAGGCCGAA AUCCACAU
1760 UGGGCAA CUGAUGAGGCCGAAAGGCCGAA AAUCCACA
1762 AAUGGGCA CUGAUGAGGCCGAAAGGCCGAA AUAAUCCA
1763 UAAUGGGC CUGAUGAGGCCGAAAGGCCGAA AAUAAUCC
1770 UAUUAAU CUGAUGAGGCCGAAAGGCCGAA AUGGGCAA
1771 UUAUAAA CUGAUGAGGCCGAAAGGCCGAA AAUGGGCA
1773 CUUUAUUA CUGAUGAGGCCGAAAGGCCGAA AUAAUGGG
1774 UCUUUAU CUGAUGAGGCCGAAAGGCCGAA AAUAAUGG
1775 CUCUUUAU CUGAUGAGGCCGAAAGGCCGAA AAAUAAUG
1778 AUCCUCU CUGAUGAGGCCGAAAGGCCGAA AUUAAUA
1787 AAUUGACA CUGAUGAGGCCGAAAGGCCGAA AUCCUCU

Table AVIII: Human Stromelysin Hairpin Ribozyme and Target Sequences

nt. Position	RZ	Substrate
66	CCACAGC AGAA GUAGGA ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	UCCUACU GUU GCUGUGG
82	GCTGAGCA AGAA GCCACG ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	CGUGGCA GUU UGCUACG
91	AAUGGAUA AGAA GAGCAA ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	UUGCUCA GCC UAUCCAUU
192	UCCUAACA AGAA GUUUA ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	UGAAACA GUU UGUUAGGA
220	UUUUUAC AGAA GGACCA ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	UGGUCCU GUU GUUAAAA
328	UGACCAAC AGAA GGAACU ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	AGUUCU GAU GUUGUCA
412	UUUGGCAA AGAA GGUGUA ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	UACACCA GAU UUGCCAAA
430	GCAGAAUC AGAA GCAUCU ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	AGAUGCU GUU GAUUCUG
439	UUCUAAAC AGAA GAUUA ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	UGAUUCU GCU GUUGAGAA
442	GCUUUCUC AGAA GCAGAA ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	UUCUGCU GUU GAGAAAGC
691	AUUCUAUG AGAA GCAACG ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	CGUUGCU GCU CAUGAAAU
775	CGAGUCAG AGAA GUGAGU ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	ACUCACA GAC CUGACUCG
780	GGAAACGA AGAA GGUCUG ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	CAGACCU GAC UCGGUUCC
786	ACAGGCGG AGAA GAGUCA ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	UGACUCG GUU CCGCCUGU
791	UUGAGACA AGAA GAACCG ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	CGGUUCC GCC UGUCUCA
795	CAUCUUGA AGAA GCGGGA ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	UCGGCCU GUC UCAAGAU
822	CAUAGAGG AGAA GAAUGC ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	GCAUUAU GUC CCUCUAUG
844	UCAGGGGA AGAA GGGGGA ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	UCCCCCU GAC UCCCTUGA
880	UCUGGAGG AGAA GGUUCC ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	GGAAACU GUC CCUCACGA
919	AAGGACAA AGAA GGAUCA ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	UGAUCCU GCU UUGUCCUU
963	CUUUAAAG AGAA GGAUUU ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	AAAUCCU GAU CUUUAAG
1360	UCTUCAA AGAA GCAUCA ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	UGAUCCU GUU UUUGAAGA
1407	CAAACUCC AGAA GUGAAG ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	CUUCACA GUU GGAGUUUG
1460	AUUAAGCC AGAA GUUACU ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	AGUAACA GCU GGCUUAAU
1570	ACAGCACA AGAA GGAGAA ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	UUUCUCCU GCC UGUGUCGU
1667	AGCUAAGC AGAA GCCCAU ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	AUGGGCU GCU GCUUAGCU
1670	GCAAGCTA AGAA GCAGCC ACCAGAGAAAACACACGUGUGUGGUAUAUUAUACUGGUA	GGCUGCU GCU UAGCUUGC

Table AIX: Rabbit Hairpin Ribozyme and Target Sequences

nt. Position	Ribozyme Sequence	Substrate
77	CCAGCCAC AGAA GUAGCA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UGCUACU GCU GUGGUGG
99	AGUGGUA AGAA GAGCAA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UUGCUCA GCC UAUCCACU
203	UUUUAACA AGAA GUUUA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UGAACA GUU UGUUAAAA
231	UUUUUAC AGAA GGAUA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UAGUCCU GUU GUUAAAA
339	UGACCAAC AGAA GGAAG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CGUUCU GAU GUUGUCA
423	CUUGCCAG AGAA GGUGG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CACACG GAU CUGCCAG
441	GCAGCAUC AGAA GCAUC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	AGAUCCU GUU GAUGUCC
702	AGCUCAUG AGAA GCAACA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UGUCCU GCU CAUGAGCU
731	CCAGUGA AGAA GACCA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UGGUCU GUU UACUCCG
758	CUGGUAC AGAA GGGUU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	AGGUCU GAU GUACCCAG
768	GCGUUGA AGAA GGGUAC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	GUACCA GUC UACAAACG
786	CGGCCAG AGAA GUGAG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CUACCA GAC CUGGCCG
797	AAAGCCG AGAA GGGCA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UGGCCG GUU CCGCCUU
802	UUAGAA AGAA GAAAG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CGGUCC GCC UUUUCAA
849	GAGCCAG AGAA GGUCA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UGGACG GCC CUGCCUC
855	UCAGAGA AGAA GGGCC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	GGCCCU GCC UCUCCUA
891	CCUGAGG AGAA GGUCC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	GGACCU GUC CUCUCCAG
930	AGGACAG AGAA GGAUA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UGAUCA GAU CUGUCCU
935	CAUGAG AGAA GAUCG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CAGUCU GUC CUUGAUG
974	CUUAAAG AGAA GAUUU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	AAAUUU GUU CUUUNAG
1107	AAAUAGA AGAA GUUUC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	GGAUACU GUU UUCAUUU
1130	UGGCCAG AGAA GAGUC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	GAAUCA GUU CUGGCCA
1301	GUCCAGG AGAA GUCUC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	AGAGACA GUC CCUGGAC
1371	GUUCAA AGAA GCAUC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CGAUCCU GUU UUGAAGC
1418	CAACUCC AGAA GUGAG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CUACCA GUC GGAGUUG
1471	CUAAAAC AGAA GUUCU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	AGCAACA GCU GGUUCAG
1663	AGCUAAGC AGAA GCUUG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	ACAGGU GCU GCUUAGCU
1666	GCAAGUA AGAA GCAGC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	GGUCCU GCU UAGCUCC
1733	AUACUUC AGAA GUCACA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UGAGACA GAC GAGUGAUU

Table BII: Human B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	AAACCCU C UGUAAAG	236	UGUGUGU U UUGUAAA
12	CCUCUGU A AAGUAAC	237	GUGUGUU U UGUAAAC
17	GUAAGU A ACAGAAG	238	UGUGUUU U GUAAACA
26	CAGAAGU U AGAAGGG	241	GUUUUGU A AACAUCA
27	AGAAGUU A GAAGGGG	247	UAAACAU C ACUGGAG
41	GAAUGU C GCCUCUC	258	GGAGGGU C UUCUACG
46	GUCGCCU C UCUGAAG	260	AGGGUCU U CUACGUG
48	CGCCUCU C UGAAGAU	261	GGGUCUU C UACGUGA
56	UGAAGAU U ACCCAA	263	GUCUUCU A CGUGAGC
57	GAAGAUU A CCCAAAG	274	GAGCAAU U GGAUUGU
75	AAGUGAU U UGUCAUU	279	AUUGGAU U GUCAUCA
76	AGUGAUU U GUCAUUG	282	GGAUUGU C AUCAGCC
79	GAUUUGU C AUUGCUU	285	UUGUCAU C AGCCUCG
82	UUGUCAU U GCUUUAU	298	UGCCUGU U UUGCACC
86	CAUUGCU U UAUAGAC	299	GCCUGUU U UGCACCU
87	AUUGCUU U AUAGACU	300	CCUGUUU U GCACCUG
88	UUGCUUU A UAGACUG	322	CCCUGGU C UUACUUG
90	GCUUUAU A GACUGUA	324	CUUGGUCU U ACUUGGG
97	AGACUGU A AGAAGAG	325	UGGUCUU A CUUGGGU
110	AGAACAU C UCAGAAG	328	UCUUAU C U GGGUCCA
112	AACAUCU C AGAAGUG	333	CUUGGGU C CAAAUUG
124	GUGGAGU C UUACCCU	339	UCCAAAU U GUUGGCU
126	GGAGUCU U ACCCUGA	342	AAAUUGU U GGCUUUC
127	GAGUCUU A CCCUGAA	347	GUUGGCU U UCACUUU
137	CUGAAAU C AAAGGAU	348	UUGGCUU U CACUUUU
145	AAAGGAU U UAAAGAA	349	UGGCUUU C ACUUUUG
146	AAGGAUU U AAAGAAA	353	UUUCACU U UUGACCC
147	AGGAUUU A AAGAAAA	354	UUCACUU U UGACCCU
163	GUGGAUU U UUUCUUC	355	UCACUUU U GACCCUA
164	UGGAAUU U UUCUUCA	362	UGACCCU A AGCAUCU
165	GGAAUUU U UCUCAG	368	UAAGCAU C UGAAGCC
166	GAAUUUU U CUUCAGC	404	GGAAACU C ACCAUCC
167	AAUUUUU C UUCAGCA	410	UCACCAU C CAAGUGU
169	UUUUUCU U CAGCAAG	418	CAAGUGU C CAUACCU
170	UUUUCUU C AGCAAGC	422	UGUCCAU A CCUCAAU
187	UGAAACU A AAUCCAC	426	CAUACCU C AAUUUCU
191	ACUAAAU C CACAACC	430	CCUCAAU U UCUUUCA
200	ACAACCU U UGGAGAC	431	CUCAAUU U CUUUCAG
201	CAACCUU U GGAGACC	432	UCAAUUU C UUUCAGC
221	ACACCCU C CAAUCUC	434	AAUUUCU U UCAGCUC
226	CUCCAAU C UCUGUGU	435	AUUUCUU U CAGCUCU
228	CCAAUCU C UGUGUGU	436	UUUCUUU C AGCUCUU

441	UUCAGCU C UUGGUGC	782	GUGACGU U AUCAGUC
443	CAGCUCU U GGUGCUG	783	UGACGUU A UCAGUCA
457	GGCUGGU C UUUUCA	785	ACGUUUAU C AGUCAAA
459	CUGGUCU U UCUCACU	789	UAUCAGU C AAAGCUG
460	UGGUCUU U CUCACUU	800	GCUGACU U CCCUACA
461	GGUCUUU C UCACUUC	801	CUGACUU C CCUACAC
463	UCUUUCU C ACUUCUG	805	CUUCCCU A CACCUAG
467	UCUCACU U CUGUUCA	811	UACACCU A GUUAUUC
468	CUCACUU C UGUUCAG	814	ACCUAGU A UAUCUGA
472	CUUCUGU U CAGGUGU	816	CUAGUAU A UCUGACU
473	UUCUGUU C AGGUGUU	818	AGUAUAU C UGACUUU
480	CAGGUGU U AUCCACG	824	UCUGACU U UGAAAUU
481	AGGUGUU A UCCACGU	825	CUGACUU U GAAAUUC
483	GUGUUAU C CACGUGA	831	UUGAAAU U CCAACUU
521	ACGCUGU C CUGUGGU	832	UGAAAUU C CAACUUC
529	CUGUGGU C ACA AUGU	838	UCCAACU U CUAUAU
537	ACAAUGU U UCUGUUG	839	CCAACUU C UAUAUUC
538	CAAUGUU U CUGUGA	841	AACUUCU A AUUAUAG
539	AAUGUUU C UGUUGAA	844	UUCUAU A UUAGAAG
543	UUUCUGU U GAAGAGC	846	CUAAUAU U AGAAGGA
562	ACAAACU C GCAUCUA	847	UAUAUAU A GAAGGAU
567	CUCGCAU C UACUGGC	855	GAAGGAU A AUUUGCU
569	CGCAUCU A CUGGCAA	858	GGAUAAU U UGCUCAA
601	GCUGACU A UGAUGUC	859	GAUAUAU U GCUCAAC
608	AUGAUGU C UGGGGAC	863	AUUUGCU C AACUCUC
622	CAUGAAU A UAUGGCC	869	UCAACCU C UGGAGGU
624	UGAAUAU A UGGCCCG	877	UGGAGGU U UCCAGA
635	CCCGAGU A CAAGAAC	878	GGAGGUU U UCCAGAG
651	GGACCAU C UUUGAUA	879	GAGGUUU U CCAGAGC
653	ACCAUCU U UGAUAUC	880	AGGUUUU C CAGAGCC
654	CCAUCUU U GAUAUCA	889	AGAGCCU C ACCUCUC
658	CUUUGAU A UCACUAA	894	CUCACCU C UCCUGGU
660	UGAUUAU C ACUAUAU	896	CACUCUC C CUGGUUG
664	UAUCACU A AUAACCU	902	UCCUGGU U GGAAAUU
667	CACUAU A ACCUCUC	920	GAAGAAU U AAAUGCC
672	AUAACCU C UCCAUUG	921	AAGAAU A AAUGCCA
674	AACCUCU C CAUUGUG	930	AUGCCAU C AACACAA
678	UCUCCAU U GUGAUCC	942	CAACAGU U UCCCAAG
684	UUGUGAU C CUGGCUC	943	AACAGUU U CCCAAGA
691	CCUGGCU C UGCGCCC	944	ACAGUUU C CCAAGAU
701	CGCCCAU C UGACGAG	952	CCAAGAU C CUGAAAC
716	GGCACAU A CGAGUGU	966	CUGAGCU C UAUGCUG
726	AGUGUGU U GUUCUGA	968	GAGUCU A UGCUGUU
729	GUGUUGU U CUGAAGU	975	AUGCUGU U AGCAGCA
730	UGUUGUU C UGAAGUA	976	UGCUGUU A GCAGCAA
737	CUGAAGU A UGAAAAA	991	ACUGGAU U UCAUAU
751	AGACGCU U UCAAGCG	992	CUGGAUU U CAAUAUG
752	GACGCUU U CAAGCGG	993	UGGAUUU C AAUAUGA
753	ACGCUUU C AAGCGGG	997	UUUCAU A UGACAAC

1016	CACAGCU U CAUGUGU	1315	CAUGGAU C GUGGGGA
1017	ACAGCUU C AUGUGUC	1324	UGGGGAU C AUGAGGC
1024	CAUGUGU C UCAUCAA	1334	GAGGCAU U CUUCCCU
1026	UGUGUCU C AUCAAGU	1335	AGGCAU U UUCCCUU
1029	GUCUCAU C AAGUAUG	1337	GCAUUCU U CCCTUAA
1034	AUCAAGU A UGGACAU	1338	CAUUCU C CCUUAAC
1042	UGGACAU U UAAGAGU	1342	CUUCCCU U AACAAAU
1043	GGACAUU U AAGAGUG	1343	UUCCCUU A ACAAAUU
1044	GACAUUU A AGAGUGA	1350	AACAAAU U UAAGCUG
1054	AGUGAAU C AGACCUU	1351	ACAAAUU U AAGCUGU
1061	CAGACCU U CAACUGG	1352	CAAAUUU A AGCUGUU
1062	AGACCUU C AACUGGA	1359	AAGCUGU U UUACCCA
1072	CUGGAU A CAACCAA	1360	AGCUGUU U UACCCAC
1090	AGAGCAU U UUCUGA	1361	GCUGUUU U ACCCACU
1091	GAGCAU U UCCUGAU	1362	CUGUUU A CCCACUA
1092	AGCAUUU U CCUGAUA	1369	ACCCACU A CCUCACC
1093	GCAUUUU C CUGAUAA	1373	ACUACCU C ACCUUCU
1099	UCCUGAU A ACCUGCU	1378	CUCACCU U CUUAAA
1107	ACCUGCU C CCAUCCU	1379	UCACCUU C UUA AAAA
1112	CUCCCAU C CUGGGCC	1381	ACCUUCU U AAAAACC
1122	GGGCCAU U ACCUUA	1382	CCUUCU A AAAACCU
1123	GGCCAUU A CCUUAU	1390	AAAACCU C UUUCAGA
1127	AUUACCU U AAUCUCA	1392	AACCUCU U UCAGAUU
1128	UUACCUU A AUCUCAG	1393	ACCUCU U CAGAUUA
1131	CCUUAU C UCAGUAA	1394	CCUCUU C AGAUUAA
1133	UUAUUCU C AGUAAAU	1399	UUCAGAU U AAGCUGA
1137	UCUCAGU A AAUGGAA	1400	UCAGAUU A AGCUGAA
1146	AUGGAU U UUUGUGA	1412	GAACAGU U ACAAGAU
1147	UGGAUUU U UUGUGAU	1413	AACAGUU A CAAGAU
1148	GGAAUUU U UGUGAUA	1429	CUGGCAU C CCUCUCC
1149	GAAUUUU U GUGAUUA	1433	CAUCCCU C UCCUUUC
1155	UUGUGAU A UGCUGCC	1435	UCCUCU C CUUUCUC
1169	CUGACCU A CUGCUUU	1438	CUCUCCU U UCUCCCC
1175	UACUGCU U UGCCCCA	1439	UCUCCUU U CUCCCCA
1176	ACUGCUU U GCCCCAA	1440	CUCCUUU C UCCCCAU
1214	GAGAGAU U GAGAAGG	1442	CCUUUCU C CCCAUU
1230	AAAGUGU A CGCCCTUG	1448	UCCCCAU A UGCAAUU
1239	GCCCTUGU A UAACAGU	1455	AUGCAAU U UGCUUAA
1241	CCUGUAU A ACAGUGU	1456	UGCAAUU U GCUUAAU
1249	ACAGUGU C CGCAGAA	1460	AUUUGCU U AAUGUAA
1275	AAAAGAU C UGAAGGU	1461	UUUGCUU A AUGUAAC
1283	UGAAGGU A GCCUCCG	1466	UUAUGU A ACCUCUU
1288	GUAGCCU C CGUCAUC	1471	GUAACCU C UUCUUUU
1292	CCUCCGU C AUCUCUU	1473	AACCUCU U CUUUUGC
1295	CCGUCAU C UCUUCUG	1474	ACCUCU C UUUUGCC
1297	GUCAUCU C UUCUGGG	1476	CUCUUCU U UUGCCAU
1299	CAUCUCU U CUGGGAU	1477	UCUUCUU U UGCCAUG
1300	AUCUCUU C UGGGAUA	1478	CUUCUUU U GCCAUGU
1307	CUGGGAU A CAUGGAU	1486	GCCAUGU U UCCAUUC

1487	CCAUGUU U CCAUUCU
1488	CAUGUUU C CAUUCUG
1492	UUUCCAU U CUGCCAU
1493	UUCCAU U UGCCAUC
1500	CUGCCAU C UUGAAU
1502	GCCAUCU U GAAUUGU
1507	CUUGAAU U GUCUUGU
1510	GAAUUGU C UUGUCAG
1512	AUUGUCU U GUCAGCC
1515	GUCUUGU C AGCCAAU
1523	AGCCAAU U CAUUAUC
1524	GCCAAU C AUUAUCU
1527	AAUUCAU U AUCUAU
1528	AUUCAU A UCUAUA
1530	UCAUAU C UAUUAAA
1532	AUUAUCU A UUAACA
1534	UAUCUAU U AAACACU
1535	AUCUAU A AACACUA
1542	AAACACU A AUUGAG

Table BIII: Human B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
8	CUUUACA CUGAUGAGGCCGAAAGGCCGAA AGGGUUU
12	GUUACUU CUGAUGAGGCCGAAAGGCCGAA ACAGAGG
17	CUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACUUUAC
26	CCCUUCU CUGAUGAGGCCGAAAGGCCGAA ACUUCUG
27	CCCCUUC CUGAUGAGGCCGAAAGGCCGAA AACUUCU
41	GAGAGGC CUGAUGAGGCCGAAAGGCCGAA ACAUUUC
46	CUUCAGA CUGAUGAGGCCGAAAGGCCGAA AGGCGAC
48	AUCUUCA CUGAUGAGGCCGAAAGGCCGAA AGAGGCG
56	UUUGGGU CUGAUGAGGCCGAAAGGCCGAA AUCUUCA
57	CUUUGGG CUGAUGAGGCCGAAAGGCCGAA AAUCUUC
75	AAUGACA CUGAUGAGGCCGAAAGGCCGAA AUCACUU
76	CAAUGAC CUGAUGAGGCCGAAAGGCCGAA AAUCACU
79	AAGCAAU CUGAUGAGGCCGAAAGGCCGAA ACAAUUC
82	AUAAAGC CUGAUGAGGCCGAAAGGCCGAA AUGACAA
86	GUCUAUA CUGAUGAGGCCGAAAGGCCGAA AGCAAUG
87	AGUCUAU CUGAUGAGGCCGAAAGGCCGAA AAGCAAU
88	CAGUCUA CUGAUGAGGCCGAAAGGCCGAA AAAGCAA
90	UACAGUC CUGAUGAGGCCGAAAGGCCGAA AUAAAGC
97	CUCUUCU CUGAUGAGGCCGAAAGGCCGAA ACAGUCU
110	CUUCUGA CUGAUGAGGCCGAAAGGCCGAA AUGUUCU
112	CACUUCU CUGAUGAGGCCGAAAGGCCGAA AGAUGUU
124	AGGGUAA CUGAUGAGGCCGAAAGGCCGAA ACUCCAC
126	UCAGGGU CUGAUGAGGCCGAAAGGCCGAA AGACUCC
127	UUCAGGG CUGAUGAGGCCGAAAGGCCGAA AAGACUC
137	AUCCUUU CUGAUGAGGCCGAAAGGCCGAA AUUUCAG
145	UUCUUUA CUGAUGAGGCCGAAAGGCCGAA AUCCUUU
146	UUUCUUU CUGAUGAGGCCGAAAGGCCGAA AAUCCUU
147	UUUUCUU CUGAUGAGGCCGAAAGGCCGAA AAAUCCU
163	GAAGAAA CUGAUGAGGCCGAAAGGCCGAA AUUCCAC
164	UGAAGAA CUGAUGAGGCCGAAAGGCCGAA AAUCCA
165	CUGAAGA CUGAUGAGGCCGAAAGGCCGAA AAUUCC
166	GCUGAAG CUGAUGAGGCCGAAAGGCCGAA AAAAUUC
167	UGCUGAA CUGAUGAGGCCGAAAGGCCGAA AAAAAUU
169	CUUGCTU CUGAUGAGGCCGAAAGGCCGAA AGAAAAA
170	GCUUGCU CUGAUGAGGCCGAAAGGCCGAA AAGAAAA
187	GUGGAUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
191	GGUUGUG CUGAUGAGGCCGAAAGGCCGAA AUUUAGU
200	GUCUCCA CUGAUGAGGCCGAAAGGCCGAA AGGUUGU
201	GGUCUCC CUGAUGAGGCCGAAAGGCCGAA AAGGUUG
221	GAGAUUG CUGAUGAGGCCGAAAGGCCGAA AGGGUGU
226	ACACAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGAG

228 ACACACA CUGAUGAGGCCGAAAGGCCGAA AGAUUGG
236 UUUACAA CUGAUGAGGCCGAAAGGCCGAA ACACACA
237 GUUUACA CUGAUGAGGCCGAAAGGCCGAA AACACAC
238 UGUUUAC CUGAUGAGGCCGAAAGGCCGAA AAACACA
241 UGAUGUU CUGAUGAGGCCGAAAGGCCGAA ACAAAC
247 CUCCAGU CUGAUGAGGCCGAAAGGCCGAA AUGUUUA
258 CGUAGAA CUGAUGAGGCCGAAAGGCCGAA ACCCUCC
260 CACGUAG CUGAUGAGGCCGAAAGGCCGAA AGACCCU
261 UCACGUA CUGAUGAGGCCGAAAGGCCGAA AAGACCC
263 GCUCACG CUGAUGAGGCCGAAAGGCCGAA AGAAGAC
274 ACAAUCC CUGAUGAGGCCGAAAGGCCGAA AUUGCUC
279 UGAUGAC CUGAUGAGGCCGAAAGGCCGAA AUCCAAU
282 GGCUGAU CUGAUGAGGCCGAAAGGCCGAA ACAAUCC
285 CAGGGCU CUGAUGAGGCCGAAAGGCCGAA AUGACAA
298 GGUGCAA CUGAUGAGGCCGAAAGGCCGAA ACAGGCA
299 AGGUGCA CUGAUGAGGCCGAAAGGCCGAA AACAGGC
300 CAGGUGC CUGAUGAGGCCGAAAGGCCGAA AAACAGG
322 CAAGUAA CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
324 CCCAAGU CUGAUGAGGCCGAAAGGCCGAA AGACCA
325 ACCCAAG CUGAUGAGGCCGAAAGGCCGAA AAGACCA
328 UGGACCC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
333 CAUUUUG CUGAUGAGGCCGAAAGGCCGAA ACCCAAG
339 AGCCAAC CUGAUGAGGCCGAAAGGCCGAA AUUUGGA
342 GAAAGCC CUGAUGAGGCCGAAAGGCCGAA ACAAUUU
347 AAAGUGA CUGAUGAGGCCGAAAGGCCGAA AGCCAAC
348 AAAAGUG CUGAUGAGGCCGAAAGGCCGAA AAGCCAA
349 CAAAAGU CUGAUGAGGCCGAAAGGCCGAA AAAGCCA
353 GGGUCAA CUGAUGAGGCCGAAAGGCCGAA AGUGAAA
354 AGGGUCA CUGAUGAGGCCGAAAGGCCGAA AAGUGAA
355 UAGGGUC CUGAUGAGGCCGAAAGGCCGAA AAAGUGA
362 AGAUGCU CUGAUGAGGCCGAAAGGCCGAA AGGGUCA
368 GGCUUCA CUGAUGAGGCCGAAAGGCCGAA AUGCUUA
404 GGAUGGU CUGAUGAGGCCGAAAGGCCGAA AUGUUCU
410 ACACUUG CUGAUGAGGCCGAAAGGCCGAA AUGGUGA
418 AGGUUUG CUGAUGAGGCCGAAAGGCCGAA ACACUUG
422 AUUGAGG CUGAUGAGGCCGAAAGGCCGAA AUGGACA
426 AGAAAUU CUGAUGAGGCCGAAAGGCCGAA AGGUUUG
430 UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AUUGAGG
431 CUGAAAG CUGAUGAGGCCGAAAGGCCGAA AAUUGAG
432 GCUGAAA CUGAUGAGGCCGAAAGGCCGAA AAAUUGA
434 GAGCUGA CUGAUGAGGCCGAAAGGCCGAA AGAAAUU
435 AGAGCUG CUGAUGAGGCCGAAAGGCCGAA AAGAAAU
436 AAGAGCU CUGAUGAGGCCGAAAGGCCGAA AAAGAAA
441 GCACCAA CUGAUGAGGCCGAAAGGCCGAA AGCUGAA
443 CAGCACC CUGAUGAGGCCGAAAGGCCGAA AGAGCUG
457 UGAGAAA CUGAUGAGGCCGAAAGGCCGAA ACCAGCC
459 AGUGAGA CUGAUGAGGCCGAAAGGCCGAA AGACCAG
460 AAGUGAG CUGAUGAGGCCGAAAGGCCGAA AAGACCA
461 GAAGUGA CUGAUGAGGCCGAAAGGCCGAA AAAGACC

463 CAGAAGU CUGAUGAGGCCGAAAGGCCGAA AGAAAGA
467 UGAACAG CUGAUGAGGCCGAAAGGCCGAA AGUGAGA
468 CUGAACA CUGAUGAGGCCGAAAGGCCGAA AAGUGAG
472 ACACCTUG CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
473 AACACCU CUGAUGAGGCCGAAAGGCCGAA AACAGAA
480 CGUGGAU CUGAUGAGGCCGAAAGGCCGAA ACACCTUG
481 ACGUGGA CUGAUGAGGCCGAAAGGCCGAA AACACCU
483 UCACGUG CUGAUGAGGCCGAAAGGCCGAA AUAACAC
521 ACCACAG CUGAUGAGGCCGAAAGGCCGAA ACAGCGU
529 ACAUUGU CUGAUGAGGCCGAAAGGCCGAA ACCACAG
537 CAACAGA CUGAUGAGGCCGAAAGGCCGAA ACAUUGU
538 UCAACAG CUGAUGAGGCCGAAAGGCCGAA AACAUUG
539 UUCAACA CUGAUGAGGCCGAAAGGCCGAA AAACAUU
543 GCUCUUC CUGAUGAGGCCGAAAGGCCGAA ACAGAAA
562 UAGAUGC CUGAUGAGGCCGAAAGGCCGAA AGUUUGU
567 GCCAGUA CUGAUGAGGCCGAAAGGCCGAA AUGCGAG
569 UUGCCAG CUGAUGAGGCCGAAAGGCCGAA AGAUGCG
601 GACAUCA CUGAUGAGGCCGAAAGGCCGAA AGUCAGC
608 GUCCCCA CUGAUGAGGCCGAAAGGCCGAA ACAUCAU
622 GGCCAUU CUGAUGAGGCCGAAAGGCCGAA AUUCAUG
624 CGGGCCA CUGAUGAGGCCGAAAGGCCGAA AUAUUCA
635 GUUCUUG CUGAUGAGGCCGAAAGGCCGAA ACUCGGG
651 UAUCAAA CUGAUGAGGCCGAAAGGCCGAA AUGGUCC
653 GAUAUCA CUGAUGAGGCCGAAAGGCCGAA AGAUGGU
654 UGAUAUC CUGAUGAGGCCGAAAGGCCGAA AAGAUGG
658 UUAGUGA CUGAUGAGGCCGAAAGGCCGAA AUCAAAG
660 UAUUAGU CUGAUGAGGCCGAAAGGCCGAA AUAUCAA
664 AGGUUAU CUGAUGAGGCCGAAAGGCCGAA AGUGAUA
667 GAGAGGU CUGAUGAGGCCGAAAGGCCGAA AUUAGUG
672 CAAUGGA CUGAUGAGGCCGAAAGGCCGAA AGGUUAU
674 CACAAUG CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
678 GGAUCAC CUGAUGAGGCCGAAAGGCCGAA AUGGAGA
684 GAGCCAG CUGAUGAGGCCGAAAGGCCGAA AUCACAA
691 GGGCGCA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
701 CUCGUCA CUGAUGAGGCCGAAAGGCCGAA AUGGGCG
716 ACACUCG CUGAUGAGGCCGAAAGGCCGAA AUGUGCC
726 UCAGAAC CUGAUGAGGCCGAAAGGCCGAA ACACACU
729 ACTUCAG CUGAUGAGGCCGAAAGGCCGAA ACAACAC
730 UACTUCA CUGAUGAGGCCGAAAGGCCGAA AACAAAC
737 UUUUUCA CUGAUGAGGCCGAAAGGCCGAA ACUUCAG
751 CGCUUGA CUGAUGAGGCCGAAAGGCCGAA AGCGUCU
752 CCGCUUG CUGAUGAGGCCGAAAGGCCGAA AAGCGUC
753 CCCGCUU CUGAUGAGGCCGAAAGGCCGAA AAAGCGU
782 GACUGAU CUGAUGAGGCCGAAAGGCCGAA ACGUCAC
783 UGACUGA CUGAUGAGGCCGAAAGGCCGAA AACGUCA
785 UUUGACU CUGAUGAGGCCGAAAGGCCGAA AUAACGU
789 CAGCUUU CUGAUGAGGCCGAAAGGCCGAA ACUGAUA
800 UGUAGGG CUGAUGAGGCCGAAAGGCCGAA AGUCAGC
801 GUGUAGG CUGAUGAGGCCGAAAGGCCGAA AAGUCAG

805 CUAGGUG CUGAUGAGGCCGAAAGGCCGAA AGGGAAG
811 GAUAUAC CUGAUGAGGCCGAAAGGCCGAA AGGUGUA
814 UCAGAUU CUGAUGAGGCCGAAAGGCCGAA ACTUAGGU
816 AGUCAGA CUGAUGAGGCCGAAAGGCCGAA AUACUAG
818 AAAGUCA CUGAUGAGGCCGAAAGGCCGAA AUUAUACU
824 AAUUUCA CUGAUGAGGCCGAAAGGCCGAA AGUCAGA
825 GAAUUUC CUGAUGAGGCCGAAAGGCCGAA AAGUCAG
831 AAGUUGG CUGAUGAGGCCGAAAGGCCGAA AUUUCAA
832 GAAGUUG CUGAUGAGGCCGAAAGGCCGAA AAUUUCA
838 AUAUUAG CUGAUGAGGCCGAAAGGCCGAA AGUUUGA
839 AAUAUUA CUGAUGAGGCCGAAAGGCCGAA AAGUUGG
841 CUAUAUA CUGAUGAGGCCGAAAGGCCGAA AGAAGUU
844 CUUCUAA CUGAUGAGGCCGAAAGGCCGAA AUUAGAA
846 UCCUUCU CUGAUGAGGCCGAAAGGCCGAA AUUUUAG
847 AUCCUUC CUGAUGAGGCCGAAAGGCCGAA AAUAUUA
855 AGCAAAU CUGAUGAGGCCGAAAGGCCGAA AUCCUUC
858 UUGAGCA CUGAUGAGGCCGAAAGGCCGAA AUUAUCC
859 GUUGAGC CUGAUGAGGCCGAAAGGCCGAA AAUAUUC
863 AGAGGUU CUGAUGAGGCCGAAAGGCCGAA AGCAAAU
869 ACCUCCA CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
877 UCUGGAA CUGAUGAGGCCGAAAGGCCGAA ACCUCCA
878 CUCUGGA CUGAUGAGGCCGAAAGGCCGAA AACCUCC
879 GCUCUGG CUGAUGAGGCCGAAAGGCCGAA AAACCTUC
880 GGCUCUG CUGAUGAGGCCGAAAGGCCGAA AAAACCU
889 GAGAGGU CUGAUGAGGCCGAAAGGCCGAA AGGCTUCU
894 ACCAGGA CUGAUGAGGCCGAAAGGCCGAA AGGUGAG
896 CAACCAG CUGAUGAGGCCGAAAGGCCGAA AGAGGUG
902 AUUUUCC CUGAUGAGGCCGAAAGGCCGAA ACCAGGA
920 GGCAUUU CUGAUGAGGCCGAAAGGCCGAA AUUCUUC
921 UGGCAUU CUGAUGAGGCCGAAAGGCCGAA AAUUCUU
930 UUGUGUU CUGAUGAGGCCGAAAGGCCGAA AUGGCAU
942 CUUGGGA CUGAUGAGGCCGAAAGGCCGAA ACUGUUG
943 UCUUGGG CUGAUGAGGCCGAAAGGCCGAA AACUGUU
944 AUCUUGG CUGAUGAGGCCGAAAGGCCGAA AAACUGU
952 GUUUCAG CUGAUGAGGCCGAAAGGCCGAA AUCUUGG
966 CAGCAUA CUGAUGAGGCCGAAAGGCCGAA AGCUCAG
968 AACAGCA CUGAUGAGGCCGAAAGGCCGAA AGAGCUC
975 UGCUGCU CUGAUGAGGCCGAAAGGCCGAA ACAGCAU
976 UUGCUGC CUGAUGAGGCCGAAAGGCCGAA AACAGCA
991 AUAUUGA CUGAUGAGGCCGAAAGGCCGAA AUCCAGU
992 CAUAUUG CUGAUGAGGCCGAAAGGCCGAA AAUCCAG
993 UCAUAUU CUGAUGAGGCCGAAAGGCCGAA AAUCCA
997 GUUGUCA CUGAUGAGGCCGAAAGGCCGAA AUUGAAA
1016 ACACAUG CUGAUGAGGCCGAAAGGCCGAA AGCUGUG
1017 GACACAU CUGAUGAGGCCGAAAGGCCGAA AAGCUGU
1024 UUGAUGA CUGAUGAGGCCGAAAGGCCGAA ACACAUG
1026 ACUUGAU CUGAUGAGGCCGAAAGGCCGAA AGACACA
1029 CAUACUU CUGAUGAGGCCGAAAGGCCGAA AUGAGAC
1034 AUGUCCA CUGAUGAGGCCGAAAGGCCGAA ACUUGAU

1042	ACUCUUA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCCA
1043	CACUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAUGUCC
1044	UCACUCU	CUGAUGAGGCCGAAAGGCCGAA	AAAUGUC
1054	AAGGUCU	CUGAUGAGGCCGAAAGGCCGAA	AUUCACU
1061	CCAGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCUG
1062	UCCAGUU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUCU
1072	UUGGUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCAG
1090	UCAGGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGCUCU
1091	AUCAGGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGCUC
1092	UAUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AAAUGCU
1093	UUAUCAG	CUGAUGAGGCCGAAAGGCCGAA	AAAAUGC
1099	AGCAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUCAGGA
1107	AGGAUGG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGGU
1112	GGCCCAG	CUGAUGAGGCCGAAAGGCCGAA	AUGGGAG
1122	UUAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AUGGGCC
1123	AUUAAGG	CUGAUGAGGCCGAAAGGCCGAA	AAUGGCC
1127	UGAGAUU	CUGAUGAGGCCGAAAGGCCGAA	AGGUAAU
1128	CUGAGAU	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAA
1131	UUACUGA	CUGAUGAGGCCGAAAGGCCGAA	AUUAAGG
1133	AUUUACU	CUGAUGAGGCCGAAAGGCCGAA	AGAUUAA
1137	UUCCAUU	CUGAUGAGGCCGAAAGGCCGAA	ACUGAGA
1146	UCACAAA	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAU
1147	AUCACAA	CUGAUGAGGCCGAAAGGCCGAA	AAUUCCA
1148	UAUCACA	CUGAUGAGGCCGAAAGGCCGAA	AAAUUCC
1149	AUAUCAC	CUGAUGAGGCCGAAAGGCCGAA	AAAUAUC
1155	GGCAGCA	CUGAUGAGGCCGAAAGGCCGAA	AUCACAA
1169	AAAGCAG	CUGAUGAGGCCGAAAGGCCGAA	AGGUCAG
1175	UGGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCAGUA
1176	UUGGGGC	CUGAUGAGGCCGAAAGGCCGAA	AAGCAGU
1214	CCUUCUC	CUGAUGAGGCCGAAAGGCCGAA	AUCUCUC
1230	CAGGGCG	CUGAUGAGGCCGAAAGGCCGAA	ACACUUU
1239	ACUGUUA	CUGAUGAGGCCGAAAGGCCGAA	ACAGGGC
1241	ACACUGU	CUGAUGAGGCCGAAAGGCCGAA	AUACAGG
1249	UUCUGCG	CUGAUGAGGCCGAAAGGCCGAA	ACACUGU
1275	ACCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUU
1283	CGGAGGC	CUGAUGAGGCCGAAAGGCCGAA	ACCUUCA
1288	GAUGACG	CUGAUGAGGCCGAAAGGCCGAA	AGGCUAC
1292	AAGAGAU	CUGAUGAGGCCGAAAGGCCGAA	ACGGAGG
1295	CAGAAGA	CUGAUGAGGCCGAAAGGCCGAA	AUGACGG
1297	CCCAGAA	CUGAUGAGGCCGAAAGGCCGAA	AGAUGAC
1299	AUCCCAG	CUGAUGAGGCCGAAAGGCCGAA	AGAGAUG
1300	UAUCCCA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGAU
1307	AUCCAUG	CUGAUGAGGCCGAAAGGCCGAA	AUCCCAG
1315	UCCCCAC	CUGAUGAGGCCGAAAGGCCGAA	AUCCAUG
1324	GCCUCAU	CUGAUGAGGCCGAAAGGCCGAA	AUCCCCA
1334	AGGGAAG	CUGAUGAGGCCGAAAGGCCGAA	AUGCCUC
1335	AAGGGAA	CUGAUGAGGCCGAAAGGCCGAA	AAUGCCU
1337	UUAAGGG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUC
1338	GUUAAGG	CUGAUGAGGCCGAAAGGCCGAA	AAGAAUG

1342 AUUUGUU CUGAUGAGGCCGAAAGGCCGAA AGGGAAG
1343 AAUUGU CUGAUGAGGCCGAAAGGCCGAA AAGGGAA
1350 CAGCUUA CUGAUGAGGCCGAAAGGCCGAA AUUUGUU
1351 ACAGCUU CUGAUGAGGCCGAAAGGCCGAA AAUUGU
1352 AACAGCU CUGAUGAGGCCGAAAGGCCGAA AAUUG
1359 UGGGUA CUGAUGAGGCCGAAAGGCCGAA ACAGCUU
1360 GUGGUA CUGAUGAGGCCGAAAGGCCGAA AACAGCU
1361 AGUGGU CUGAUGAGGCCGAAAGGCCGAA AAACAGC
1362 UAGUGG CUGAUGAGGCCGAAAGGCCGAA AAAACAG
1369 GGUGAG CUGAUGAGGCCGAAAGGCCGAA AGUGGU
1373 AGAAGU CUGAUGAGGCCGAAAGGCCGAA AGGUAGU
1378 UUUUAG CUGAUGAGGCCGAAAGGCCGAA AGGUGAG
1379 UUUUAA CUGAUGAGGCCGAAAGGCCGAA AAGGUGA
1381 GGUUUU CUGAUGAGGCCGAAAGGCCGAA AGAAGGU
1382 AGGUUU CUGAUGAGGCCGAAAGGCCGAA AAGAAGG
1390 UCUGAA CUGAUGAGGCCGAAAGGCCGAA AGGUUU
1392 AAUCUGA CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
1393 UAAUCUG CUGAUGAGGCCGAAAGGCCGAA AAGAGGU
1394 UUAUCU CUGAUGAGGCCGAAAGGCCGAA AAAGAGG
1399 UCAGCUU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA
1400 UUCAGCU CUGAUGAGGCCGAAAGGCCGAA AAUCUGA
1412 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA ACUGUUC
1413 CAUCUUG CUGAUGAGGCCGAAAGGCCGAA AACUGUU
1429 GGAGAG CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
1433 GAAAGGA CUGAUGAGGCCGAAAGGCCGAA AGGAUG
1435 GAGAAAG CUGAUGAGGCCGAAAGGCCGAA AGAGGA
1438 GGGGAG CUGAUGAGGCCGAAAGGCCGAA AGGAGAG
1439 UGGGAG CUGAUGAGGCCGAAAGGCCGAA AAGGAG
1440 AUGGGA CUGAUGAGGCCGAAAGGCCGAA AAAGGAG
1442 AUAUGG CUGAUGAGGCCGAAAGGCCGAA AGAAAGG
1448 AAUUGCA CUGAUGAGGCCGAAAGGCCGAA AUGGGA
1455 UUAAGCA CUGAUGAGGCCGAAAGGCCGAA AUUGCAU
1456 AUUAAGC CUGAUGAGGCCGAAAGGCCGAA AAUUGCA
1460 UUAACAU CUGAUGAGGCCGAAAGGCCGAA AGCAAU
1461 GUUACAU CUGAUGAGGCCGAAAGGCCGAA AAGCAA
1466 AAGAGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUA
1471 AAAAGAA CUGAUGAGGCCGAAAGGCCGAA AGGUUAC
1473 GCAAAG CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
1474 GGCAAAA CUGAUGAGGCCGAAAGGCCGAA AAGAGGU
1476 AUGGCAA CUGAUGAGGCCGAAAGGCCGAA AGAAGAG
1477 CAUGGCA CUGAUGAGGCCGAAAGGCCGAA AAGAAGA
1478 ACAUGGC CUGAUGAGGCCGAAAGGCCGAA AAAGAAG
1486 GAAUGGA CUGAUGAGGCCGAAAGGCCGAA ACAUGGC
1487 AGAAUGG CUGAUGAGGCCGAAAGGCCGAA AACAUUG
1488 CAGAAUG CUGAUGAGGCCGAAAGGCCGAA AAACAUG
1492 AUGGCAG CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
1493 GAUGGCA CUGAUGAGGCCGAAAGGCCGAA AAUGGAA
1500 AAUUCAA CUGAUGAGGCCGAAAGGCCGAA AUGGCAG
1502 ACAAUUC CUGAUGAGGCCGAAAGGCCGAA AGAUGGC

1507	ACAAGAC	CUGAUGAGGCCGAAAGGCCGAA	AUUCAAG
1510	CUGACAA	CUGAUGAGGCCGAAAGGCCGAA	ACAAUUC
1512	GGCUGAC	CUGAUGAGGCCGAAAGGCCGAA	AGACAAU
1515	AUUGGCU	CUGAUGAGGCCGAAAGGCCGAA	ACAAGAC
1523	GAUAAUG	CUGAUGAGGCCGAAAGGCCGAA	AUUGGCU
1524	AGAUAAU	CUGAUGAGGCCGAAAGGCCGAA	AAUUGGC
1527	AAUAGAU	CUGAUGAGGCCGAAAGGCCGAA	AUGAAUU
1528	UAAUAGA	CUGAUGAGGCCGAAAGGCCGAA	AAUGAAU
1530	UUUAAUA	CUGAUGAGGCCGAAAGGCCGAA	AUAAUGA
1532	UGUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AGAUAAU
1534	AGUGUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAGAUU
1535	UAGUGUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAGAU
1542	CUCAAAU	CUGAUGAGGCCGAAAGGCCGAA	AGUGUUU

Table BIV: Mouse B7-1 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
8	GaGUuUU a UACcUcA	108	CaUcUUU a GCAuCUG
10	guUuuAU A CCUCAAU	108	CAUcUUU a gcaUCUG
10	GUuUaU a ccuCAAU	131	aUGCCAU C caGgcUU
14	uAUacCU c aAUAGAC	142	gCUuCUU U uUCuaCA
18	CcucAAU A gaCUCUu	142	gCuUCUU u UUcUaCa
18	CCUCaaU a gaCUCUU	143	CUuCUUU u UCuaCAU
18	CcUcAAU a GaCUcuU	143	CuUcUUU u uCuAcAU
23	AuaGaCU c uUACuaG	143	CUUCUUU U uCuAcaU
25	AGACuCU U aCuAGuu	143	cUUCuUU u UCUAcau
26	GACuCUU a CuAGuuu	144	UuCuUUU U cUaCAuC
29	UCUUACU a GuuUCuc	144	UuCuuuU u cUAcAUC
29	UcUuACU a gUuuCuC	144	UUCuuUU u cuaCAUC
29	UCUuACU a guUUCUc	147	uUUUUcU a cAuCUCU
29	UCuuaCU a gUUUCUC	153	uAcAuCU C ugUUUCU
34	CUAGUuU c UCUuuuU	165	uCUCgAU U UuUgUGa
34	CUAGUuU c UCUuuuU	165	uCUcgAU U UuuGUGa
34	cUAGUuU c uCuUUUU	165	ucucgAU U UUUGUGA
40	ucuCuuU U UCAGgUU	166	CUCgAUU U uUgUGaG
41	cUCUuUU u caGGuUg	167	uCGAUuU u UGUGaGc
41	cuCUuUU U CAGgUUg	167	ucGauUU U UGUGAgC
42	uCUuUUU C AGgUUGu	167	UCgAUUU u UgUGAGC
56	UGAAACU c AAcCuuC	168	cGAUuuU u gUgAGCC
56	UGAAAcU C aAcCUUC	168	cgAUUUU U GUGAgcc
62	uCAACCU U caaAGAC	197	GCUccAU u GgCUcUA
62	UCaAcCU U CaAaGAc	202	aUUGGCU c UagaUUc
62	UCAACCU u caaAGac	208	UCuAgAU U ccUGGCU
63	CAACCUU c aaAGACa	216	CCUGGCU u UcCcCau
73	aGAcAcU c UGuUCcA	217	cUGGCUU U CcCcAUc
77	acUCUgU u cCAuUUC	217	cUgGCuU u CccCAUC
78	CucUGUU c CauUUCU	217	CUGGCuU u CCcCauC
83	UucCAuU U CUGUgga	218	UGGcuUU c ccCaUCA
93	GUggAcU A AuAGgAu	218	UGGCUUU C cCcaUca
93	gUgGacU a AUAGgaU	218	UGgCuUU c cCcaUCA
93	gUGgAcU a AuAGGAU	218	ugGcUUU c CCCAucA
96	GAcuAAU a GGAUcaU	224	UccCCAU c aUGuUCu
96	gacuAAU a gGAuCaU	224	UccCCAU c aUGuucU
101	AUaGGAU c aUCuUuA	230	UCAugUU C UccAAAg
104	GGAUCAU C uuuAgCa	232	AuGUUcU C CAaAGCa
104	GGAUCAU C UUUagca	232	AUGuUcU c caaAGCA
106	AuCAUCU U UagCAUC	232	AugUUCU c cAAAgCa
107	UcAuCuU u AGCAUCU	241	AAAGcAU c UgAAGcu
107	uCaUCUU u AgcAuCU	241	aAAGCAU C UGAAGCu

241	AAAgcAU C UGAAGcU	556	ACCuACU c uCUuAuC
249	UGAAGcU A UGGCuUG	556	AcCuAcU c ucUUAUC
264	CAAuUgU c AGuUGaU	560	AcUcUCU U aUCAuCC
287	CACCaCU c CUcaagU	561	cUCuCUU a UcAuCCU
295	CUCaAgU u UCcaUGU	561	cuCUcuU a uCAUCCU
295	cuCAaGU U UCCAUGu	561	CUCUCuU a UCauCCu
296	uCAAgUU u ccAUgUc	566	UUaUcAU C CUGGgcC
297	CAAGUuU C CAUguCc	566	uUauCAU C CUGGGCC
297	CAaGuuU c cAUGuCC	581	UGGuCcU U UcAGAcc
314	GGCUcaU u cUUCUCu	583	gucCUUU C AgaCcGG
314	GgcuCAU U CUUCuCU	583	GuCcUUU c AGAccGg
315	GcuCAUU c UuCUcuU	598	GGCACAU A CagcUGU
315	gcuCAUU C UUCuCUU	608	gcUGUGU c GUUCaaA
317	uCAUUCU U CuCUUg	611	GUGUcgU u CAaaaGA
318	CAUUCUU C uCUUgu	611	GUGUcGU U CaaAAGa
318	CAUuCuU C UCuUUGu	612	UGUcGUU C aaAAGaA
320	uUCUUCU c uuUGuGC	641	aUGaAGU u aaACaCU
320	UUCuCUU C UUhUGUC	649	AAAcacU U GGCUUa
322	CuuCUCU U uGUGCUG	649	AaaCacU U gGCUUa
322	CUucCUU u UgUGCUG	655	UUGgcUu u AGUAAAg
323	UUCuCUU u gUGcugC	656	UGgcUUU a GUAAAgU
336	gcUGAUU c GUCuUUC	659	CuUuaGU A AAGUugu
341	uUCGuCU u UCacAAG	664	GUaAaGU U gUCcaUC
341	UUCgucU u UcacAAG	667	AaGUUGU C caUCAAA
342	UcGUCUU U CaCAagU	671	UgUCcaU C AAAGCUG
343	cgucUuU C AcAAGUG	682	gCUgAcU u CuCuACC
343	cGuCuUU c AcaAGUG	682	GCUGACU U CuCUACc
352	caAGUGU C uuCAGAU	682	GCUGacU U cuCuACc
355	gUgUcUU C AGaUGUU	683	CUGACTUU C uCUACcC
382	UCcaAGU c AgUGaAA	683	CUGACTUU c ucuAccC
408	gCUGCcU U GCCguuA	685	gACUuCU c UaCCCCc
414	UUGcegU U aCAACUc	685	gaCUucU c UACCCcC
414	UUGCCgU u ACAAcUc	687	CUUCuCU A CcCCcAa
421	UaCAAcU c uCcUcAU	698	ccAACAU a ACUGagu
426	CUCuCCU c aUgAAgA	698	CCAacAU A ACuGaGU
439	GaUGAgU c UGAaGaC	718	AaCCCaU C UGcAgAc
452	acCGaAU C UACUGGC	718	aaCCCAU c UGCagac
454	CGaAUCTU A CUGGCAA	729	AGACacU A AaAgGAU
484	GuGCUgU c UGucaUU	729	agAcAcU A aaAGGAU
484	GugCUGU c UguCAuU	729	agACAcU a AaAgGAU
488	ugUcUGU C AUUGCUg	737	aaAGGAU u AccUGCU
503	gGAAacU A aaAGuGu	737	aaAGGAU U AccUGCu
503	ggAAAaU a AAagUGU	737	aaagGAU u ACCUGCU
520	CCCGAGU A uAAGAAC	745	aCCUGcU U UGCuuCc
535	cGGAcUU U aUaUGAc	745	accUGcU u UGCUuCC
536	GGAcUUU a UaUGAcA	759	cGggGgU U uCCCAA
538	AcUuUAU a UGACaac	759	cGgGGGU U UcCcAaa
553	acuACCU a cUCUcUU	759	cGGgGGU U UcCCAaA
553	AcUaCcU a cUCUcUU	760	GggGgUU u CCCAAAG

760	gGGgGUU u cCCAaag	1060	aAAUgcU u cUGUaAG
760	GGgGGUU U cCCAaAG	1060	AAAugCU u cUgUaAG
761	GgGGUUU c CCAaAGC	1061	AAUGcUU C UGUaagc
771	aAagccU C GCuUCUC	1080	AagcugU u UCAGAAG
771	AaAGCCU C gCuUCUC	1080	AAGCUGU U UcAgaag
776	CUCgCUU C UcUUGgu	1081	AgCuGUU u CAgAga
776	CUCgCuU C UCuUGGU	1121	acAGcCU U ACCuUcg
778	CgCuUCU C uUGGUUG	1121	AcAgCCU u aCCuUcG
784	UCuUGGU U GGAAAU	1121	ACagCCU u ACCUUCg
803	GAGaaUU A CCugGcA	1122	CaGcCuU a cCUUCgG
803	gAGAAUU A ccUGgCA	1126	CUuACCU u CgGgccU
803	gagAaUU a CCUGgcA	1127	UUaCcUU c ggGcCUG
812	cUGgCAU C AAuACgA	1127	UuACcUU c GggCCUg
812	CUGGcAU c aAuaCgA	1144	GaagCAU U AgCUgAA
816	caUCAAU A cGACAAU	1144	gaAGcaU u AGCUGAA
816	aUCAaU a cgACaAU	1145	aAgcAUU a GCUgAAC
824	CgACaAU U UCCCAgG	1160	AGAcCgU c UUCCUuu
825	gACaAUU U CCCAgGA	1162	AcCgUCU U CcUUuaG
826	ACAaUUU C CCAGGAU	1163	ccGUCUU c CUUuaGU
834	CCAgGAU C CUGAAuC	1167	cUUCcUU u AGuUCUU
841	CcUGaaU C ugAAUUG	1177	uUCUUCU c UguCCAU
841	cCUGAaU c UGAAuUg	1181	UCuCuGU C CAuGUGg
850	gAAuUGU A CaCCaUu	1181	ucUCUGU c CAuGUGg
869	gccAaCU a gAUuUCA	1192	gUGGGAU A CAUGGua
869	GCCaACU a GAuUuca	1199	aCaUGGU a UUAUGUG
869	GCCAAcU a gaUuUCA	1201	AuGgUaU u aUGUGGc
873	acUaGAU u UCAaUAc	1210	ugUGGcU C aUGaGGu
873	ACUaGAU U UCAAUAc	1210	UGuGGcU C AUGAGGu
874	CUaGAUU U CAUAcG	1223	GUacAAU c UUCUUu
875	UaGAUUU C AAUAcGA	1225	ACAAUcU U UCuUuca
885	UAcgACU c gcAACCa	1225	ACAAuCU u uCuUucA
899	ACACCau u aAgUgUC	1226	caAuCUU u cUuUCAG
899	ACAcCaU u AaGUGUC	1227	aAucUUU c uUUCAGC
906	UaaGUGU c UcaUuAA	1227	AAucuuU C UUCAGc
906	uAaGUGU C UCAUuAA	1227	AAuCUuU c uUucaGC
908	agUGUCU C AUuAAaU	1229	ucUUUCU U UCAGCaC
911	GUCUCAU u AAaUAUG	1230	cUUUCUU U CAGCaCc
916	AUuAaaU a UGGaGAu	1252	cUgAUCU u UcggACA
916	AUuAAaU A UGGAGAU	1274	acaAGAU a gAGuUaA
943	gAGgaCU U CAcCUGG	1310	UGAGGaU u uCuUuCc
944	AGgaCUU C AcCUGGg	1312	aGgAUUU c UuUcCAu
1001	UGCUCUU u GggGCAG	1314	gAUUUcU u UcCAuCA
1034	CAGucGU c gUCauCG	1316	UUUcUuU c CAuCAgG
1037	UcGUCgU C AuCguUG	1320	UUUcCaU C AGgAAGC
1043	uCAUCgU U GucAUCA	1320	UUUCcaU c aggaAGC
1046	ucgUUGU c AuCAUCA	1339	GgCAagU u UgCUGGG
1049	uUguCaU c AuCAAau	1355	cUuUGAU U GCUUGAU
1060	aAAUGcU U CUGUaag	1437	gUGguaU A aGAAAAA
1060	AAaUgCU u cUGUaAG	1437	gUggUAU a AGAAaaa

1475	gCCUAGU c UuaCUGc
1477	CUaGUCU U ACUgcaa
1487	ugCAaCU U gAUaUGU
1491	AcuUGAU a UGUCAUg
1491	aCUUgaU a UGuCAUG
1505	gUUUGgU U ggUGUcu
1530	uGCCcUU u uCUgAAg
1531	GCccUUU u CUGAagA
1532	CcCuUuU C UGAAGAg
1532	CcCuuuU C UGAAGAG
1644	CUaUGGU u gggAUGU
1652	ggGAuGU a AaAAcGG
1652	GgGAugU a aAaAcGG
1670	aUaAUaU a AaUAuUA
1674	uAuAAAU a UuAaaUa
1676	UaAaUAU u aAaUAAA
1677	AAauAUU a AAuaAAA
1677	AaaUAUU A AAuAaaA
1694	AGagUaU u gAGcAAA

Table BV: Mouse B7-1 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
8	UGAGGUA CUGAUGAGGCCGAAAGGCCGAA AAAACUC
10	AUUGAGG CUGAUGAGGCCGAAAGGCCGAA AUAAAAC
10	AUUGAGG CUGAUGAGGCCGAAAGGCCGAA AUAAAAC
14	GUCUAUU CUGAUGAGGCCGAAAGGCCGAA AGGUAUA
18	AAGAGUC CUGAUGAGGCCGAAAGGCCGAA AUUGAGG
18	AAGAGUC CUGAUGAGGCCGAAAGGCCGAA AUUGAGG
18	AAGAGUC CUGAUGAGGCCGAAAGGCCGAA AUUGAGG
23	CUAGUAA CUGAUGAGGCCGAAAGGCCGAA AGUCUAU
25	AACUAGU CUGAUGAGGCCGAAAGGCCGAA AGAGUCU
26	AAACUAG CUGAUGAGGCCGAAAGGCCGAA AAGAGUC
29	GAGAAAC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
29	GAGAAAC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
29	GAGAAAC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
29	GAGAAAC CUGAUGAGGCCGAAAGGCCGAA AGUAAGA
34	AAAAAGA CUGAUGAGGCCGAAAGGCCGAA AAACUAG
34	AAAAAGA CUGAUGAGGCCGAAAGGCCGAA AAACUAG
34	AAAAAGA CUGAUGAGGCCGAAAGGCCGAA AAACUAG
40	AACCUGA CUGAUGAGGCCGAAAGGCCGAA AAAGAGA
41	CAACCUG CUGAUGAGGCCGAAAGGCCGAA AAAAGAG
41	CAACCUG CUGAUGAGGCCGAAAGGCCGAA AAAAGAG
42	ACAACCU CUGAUGAGGCCGAAAGGCCGAA AAAAGA
56	GAAGGUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
56	GAAGGUU CUGAUGAGGCCGAAAGGCCGAA AGUUUCA
62	GUCUUUG CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
62	GUCUUUG CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
62	GUCUUUG CUGAUGAGGCCGAAAGGCCGAA AGGUUGA
63	UGUCUUU CUGAUGAGGCCGAAAGGCCGAA AAGGUUG
73	UGGAACA CUGAUGAGGCCGAAAGGCCGAA AGUGUCU
77	GAAAUUG CUGAUGAGGCCGAAAGGCCGAA ACAGAGU
78	AGAAUUG CUGAUGAGGCCGAAAGGCCGAA AACAGAG
83	UCCACAG CUGAUGAGGCCGAAAGGCCGAA AAUGGAA
93	AUCCUAU CUGAUGAGGCCGAAAGGCCGAA AGUCCAC
93	AUCCUAU CUGAUGAGGCCGAAAGGCCGAA AGUCCAC
93	AUCCUAU CUGAUGAGGCCGAAAGGCCGAA AGUCCAC
96	AUGAUCC CUGAUGAGGCCGAAAGGCCGAA AUUAGUC
96	AUGAUCC CUGAUGAGGCCGAAAGGCCGAA AUUAGUC
101	UAAAGAU CUGAUGAGGCCGAAAGGCCGAA AUCCUAU
104	UGCUIAA CUGAUGAGGCCGAAAGGCCGAA AUGAUCC
104	UGCUIAA CUGAUGAGGCCGAAAGGCCGAA AUGAUCC
106	GAUGCUA CUGAUGAGGCCGAAAGGCCGAA AGAUGAU

107 AGAUGCU CUGAUGAGGCCGAAAGGCCGAA AAGAUGA
107 AGAUGCU CUGAUGAGGCCGAAAGGCCGAA AAGAUGA
108 CAGAUGC CUGAUGAGGCCGAAAGGCCGAA AAAGAUG
108 CAGAUGC CUGAUGAGGCCGAAAGGCCGAA AAAGAUG
131 AAGCCUG CUGAUGAGGCCGAAAGGCCGAA AUGGCAU
142 UGUAGAA CUGAUGAGGCCGAAAGGCCGAA AAGAAGC
142 UGUAGAA CUGAUGAGGCCGAAAGGCCGAA AAGAAGC
143 AUGUAGA CUGAUGAGGCCGAAAGGCCGAA AAAGAAG
143 AUGUAGA CUGAUGAGGCCGAAAGGCCGAA AAAGAAG
143 AUGUAGA CUGAUGAGGCCGAAAGGCCGAA AAAGAAG
143 AUGUAGA CUGAUGAGGCCGAAAGGCCGAA AAAGAAG
144 GAUGUAG CUGAUGAGGCCGAAAGGCCGAA AAAAGAA
144 GAUGUAG CUGAUGAGGCCGAAAGGCCGAA AAAAGAA
144 GAUGUAG CUGAUGAGGCCGAAAGGCCGAA AAAAGAA
147 AGAGAUG CUGAUGAGGCCGAAAGGCCGAA AGAAAAA
153 AGAAACA CUGAUGAGGCCGAAAGGCCGAA AGAUGUA
165 UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUCGAGA
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167 GCUCACA CUGAUGAGGCCGAAAGGCCGAA AAAUCGA
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168 GGCUCAC CUGAUGAGGCCGAAAGGCCGAA AAAAUCG
168 GGCUCAC CUGAUGAGGCCGAAAGGCCGAA AAAAUCG
197 UAGAGCC CUGAUGAGGCCGAAAGGCCGAA AUGGAGC
202 GAAUCTA CUGAUGAGGCCGAAAGGCCGAA AGCCAAU
208 AGCCAGG CUGAUGAGGCCGAAAGGCCGAA AUCTAGA
216 AUGGGGA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
217 GAUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGCCAG
217 GAUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGCCAG
217 GAUGGGG CUGAUGAGGCCGAAAGGCCGAA AAGCCAG
218 UGAUGGG CUGAUGAGGCCGAAAGGCCGAA AAAGCCA
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264 AUCAACU CUGAUGAGGCCGAAAGGCCGAA ACAAUUG
287 ACTUGAG CUGAUGAGGCCGAAAGGCCGAA AGUGGUG
295 ACAUGGA CUGAUGAGGCCGAAAGGCCGAA ACTUGAG

295 ACAUGGA CUGAUGAGGCCGAAAGGCCGAA ACUUGAG
296 GACAUGG CUGAUGAGGCCGAAAGGCCGAA AACUUGA
297 GGACAUG CUGAUGAGGCCGAAAGGCCGAA AAACUUG
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318 ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGAAUG
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875 UCGUAUU CUGAUGAGGCCGAAAGGCCGAA AAAUCUA
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1060 CUUACAG CUGAUGAGGCCGAAAGGCCGAA AGCAUUU
1060 CUUACAG CUGAUGAGGCCGAAAGGCCGAA AGCAUUU
1061 GCUUACA CUGAUGAGGCCGAAAGGCCGAA AAGCAUU
1080 CUUCUGA CUGAUGAGGCCGAAAGGCCGAA ACAGCUU
1080 CUUCUGA CUGAUGAGGCCGAAAGGCCGAA ACAGCUU

1081	UCUUCUG	CUGAUGAGGCCGAAAGGCCGAA	AACAGCU
1121	CGAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGCTUGU
1121	CGAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGCTUGU
1121	CGAAGGU	CUGAUGAGGCCGAAAGGCCGAA	AGGCTUGU
1122	CCGAAGG	CUGAUGAGGCCGAAAGGCCGAA	AAGGCUG
1126	AGGCCCG	CUGAUGAGGCCGAAAGGCCGAA	AGGUAAG
1127	CAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAA
1127	CAGGCC	CUGAUGAGGCCGAAAGGCCGAA	AAGGUAA
1144	UUCAGCU	CUGAUGAGGCCGAAAGGCCGAA	AUGCUC
1144	UUCAGCU	CUGAUGAGGCCGAAAGGCCGAA	AUGCUC
1145	GUUCAGC	CUGAUGAGGCCGAAAGGCCGAA	AAUGCTU
1160	AAAGGAA	CUGAUGAGGCCGAAAGGCCGAA	ACGGUCU
1162	CUAAAGG	CUGAUGAGGCCGAAAGGCCGAA	AGACGGU
1163	ACUAAAG	CUGAUGAGGCCGAAAGGCCGAA	AAGACGG
1167	AAGAACU	CUGAUGAGGCCGAAAGGCCGAA	AAGGAAG
1177	AUGGACA	CUGAUGAGGCCGAAAGGCCGAA	AGAAGAA
1181	CCACAUG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAGA
1181	CCACAUG	CUGAUGAGGCCGAAAGGCCGAA	ACAGAGA
1192	UACCAUG	CUGAUGAGGCCGAAAGGCCGAA	AUCCAC
1199	CACAUAA	CUGAUGAGGCCGAAAGGCCGAA	ACCAUGU
1201	GCCACAU	CUGAUGAGGCCGAAAGGCCGAA	AUACCAU
1210	ACCUCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCCACA
1210	ACCUCAU	CUGAUGAGGCCGAAAGGCCGAA	AGCCACA
1223	AAAGAAA	CUGAUGAGGCCGAAAGGCCGAA	AUUGUAC
1225	UGAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AGAUUGU
1225	UGAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AGAUUGU
1226	CUGAAAG	CUGAUGAGGCCGAAAGGCCGAA	AAGAUUG
1227	GCUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUU
1227	GCUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUU
1227	GCUGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAGAUU
1229	GUGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AGAAAGA
1230	GGUCUG	CUGAUGAGGCCGAAAGGCCGAA	AAGAAAG
1252	UGUCCGA	CUGAUGAGGCCGAAAGGCCGAA	AGAUACG
1274	UUAACUC	CUGAUGAGGCCGAAAGGCCGAA	AUCUUGU
1310	GGAAAGA	CUGAUGAGGCCGAAAGGCCGAA	AUCCUCA
1312	AUGGAAA	CUGAUGAGGCCGAAAGGCCGAA	AAAUCCU
1314	UGAUGGA	CUGAUGAGGCCGAAAGGCCGAA	AGAAAUC
1316	CCUGAUG	CUGAUGAGGCCGAAAGGCCGAA	AAAGAAA
1320	GCUUCCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGAAA
1320	GCUUCCU	CUGAUGAGGCCGAAAGGCCGAA	AUGGAAA
1339	CCCAGCA	CUGAUGAGGCCGAAAGGCCGAA	ACUUGCC
1355	AUCAAGC	CUGAUGAGGCCGAAAGGCCGAA	AUCAAAG
1437	UUUUUCU	CUGAUGAGGCCGAAAGGCCGAA	AUACCAC
1437	UUUUUCU	CUGAUGAGGCCGAAAGGCCGAA	AUACCAC
1475	GCAGUAA	CUGAUGAGGCCGAAAGGCCGAA	ACUAGGC
1477	UUGCAGU	CUGAUGAGGCCGAAAGGCCGAA	AGACUAG
1487	ACAUUUC	CUGAUGAGGCCGAAAGGCCGAA	AGUUGCA
1491	CAUGACA	CUGAUGAGGCCGAAAGGCCGAA	AUCAAGU
1491	CAUGACA	CUGAUGAGGCCGAAAGGCCGAA	AUCAAGU

1505	AGACACC	CUGAUGAGGCCGAAAGGCCGAA	ACCAAAC
1530	CUUCAGA	CUGAUGAGGCCGAAAGGCCGAA	AAGGGCA
1531	UCUUCAG	CUGAUGAGGCCGAAAGGCCGAA	AAAGGGC
1532	CUCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGGG
1532	CUCUUCA	CUGAUGAGGCCGAAAGGCCGAA	AAAAGGG
1644	ACAUCCC	CUGAUGAGGCCGAAAGGCCGAA	ACCAUAG
1652	CCGUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCCC
1652	CCGUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACAUCCC
1670	UAAUAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUAU
1674	UAUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AUUUAUA
1676	UUUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AUAUUUA
1677	UUUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1677	UUUAUUU	CUGAUGAGGCCGAAAGGCCGAA	AAUAUUU
1694	UUUGCUC	CUGAUGAGGCCGAAAGGCCGAA	AUACUCU

Table BVI: Human B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
16	GAAAGCU U UGCUUCU	271	UAGUAGU A UUUUGGC
17	AAAGCUU U GCUUCUC	273	GUAGUUAU U UUGGCAG
21	CUUUGCU U CUCUGCU	274	UAGUUAU U UGGCAGG
22	UUUGCUU C UCUGCUG	275	AGUAUUU U GGCAGGA
24	UGCUUCU C UGUGCUU	294	GAAACU U GGUUCUG
34	CUGCUGU A ACAGGGA	298	ACUUGGU U CUGAUG
44	AGGGACU A GCACAGA	299	CUUGGUU C UGAAGA
70	GUGGGGU C AUUUGCA	310	AUGAGGU A UACUAG
73	GGGUCAU U UCCAGAU	312	GAGGUUAU A CUUAGGC
74	GGUCAUU U CCAGUA	315	GUUAUCU U AGGCAA
75	GUCAUUU C CAGUAU	316	UAUAUCU A GGCAAAG
81	UCCAGAU A UUAGGUC	330	GAGAAAU U UGACAGU
83	CAGUAU U AGGUCAC	331	AGAAAUU U GACAGUG
84	AGUAUUU A GGUCACA	340	ACAGUGU U CAUUGCA
88	AUUAGGU C ACAGCAG	341	CAGUGUU C AUUGCAA
113	AAUGGAU C CCCAGUG	344	UGUUCAU U CCAAGUA
125	GUGCAU A UGGGACU	345	GUUCAU C CAAGUAU
137	ACUGAGU A ACAUUCU	351	UCCAAGU A UAUGGGC
142	GUACAU U CUCUUUG	353	CAAGUAU A UGGGCCG
143	UAACAU C UCUUUGU	368	CACAAGU U UUGAUUC
145	ACAUUCU C UUUGUGA	369	ACAAGUU U UGAUUCG
147	AUUCUCU U UGUGAUG	370	CAAGUUU U GAUUCGG
148	UUCUCU U GUGAUGG	374	UUUGAU U CGGACAG
159	AUGGCCU U CCUGCUC	375	UUUGAUU C GGACAGU
160	UGGCCU C CUGCUCU	383	GGACAGU U GGACCUU
166	UCCUGCU C UCUGGUG	397	UGAGACU U CACAAUC
168	CUGCUCU C UGGUGCU	398	GAGACU C ACAAUUCU
179	UGCUGCU C CUCUGAA	404	UCACAAU C UUCAGAU
182	UGCUCU C UGAAGAU	406	ACAAUCU U CAGAUCA
190	UGAAGAU U CAAGCUU	407	CAAUCU C AGAUCAA
191	GAAGAU C AAGCUUA	412	UUCAGAU C AAGGACA
197	UCAAGCU U AUUCAA	426	AAGGGCU U GUUCAA
198	CAAGCUU A UUUCAU	429	GGCUUGU A UCAAUGU
200	AGCUUAU U UCAAUGA	431	CUUGUAU C AAUGUAU
201	GCUUAU U CAAUGAG	437	UCAAUGU A UCAUCCA
202	CUUAUUU C AAUGAGA	439	AAUGUAU C AUCCAUC
231	UGCCAAU U UGCAAC	442	GUUAU C CAUCACA
232	GCCAAU U GCAAAU	446	CAUCCA C AAAAAA
240	GCAAAU C UCAAAAC	469	GAAUGAU U CGCAUCC
242	AAACUCU C AAAACCA	470	AAUGAUU C GCAUCCA
265	GUGAGCU A GUAGUAU	475	UUCGAU C CACCAGA
268	AGCUAGU A GUUUUU	488	GAUGAAU U CUGAACU

489	AUGAAUU C UGAACUG	721	UGUCUGU U UCAUUCC
498	GAACUGU C AGUGCUU	722	GUCUGUU U CAUUCOC
505	CAGUGCU U GCUAACU	723	UCUGUUU C AUUCCCU
509	GCUUGCU A ACUUCAG	726	GUUUCAU U CCCUGAU
513	GCUAACU U CAGUCAA	727	UUUCAUU C CCUGAUG
514	CUAACUU C AGUCAAC	736	CUGAUGU U ACGAGCA
518	CUUCAGU C AACCTGA	737	UGAUGUU A CGAGCAA
529	CUGAAAU A GUACCAA	746	GAGCAAU A UGACCAU
532	AAAUAGU A CCAUUUU	754	UGACCAU C UUCUGUA
538	UACCAAU U UCUAUA	756	ACCAUCU U CUGUAUU
539	ACCAAUU U CUAUAU	757	CCAUCUU C UGUUUC
540	CCAUUUU C UAAUAUA	761	CUUCUGU A UUCUGGA
542	AAUUUCU A AUAUAC	763	UCUGUUA U CUGGAAA
545	UUCUAUU A UAACAGA	764	CUGUAUU C UGGAAAC
547	CUAAUAU A ACAGAAA	787	CGCGGCU U UUAUCUU
561	AAUGUGU A CAUAAAU	788	GCGGCUU U UAUCUUC
565	UGUACAU A AAUUUGA	789	CGGCUUU U AUCUUCA
569	CAUAAAU U UGACCUG	790	GGCUUUU A UCUUCAC
570	AUAAAUU U GACCUGC	792	CUUUUAU C UUCACCU
579	ACCUGCU C AUCUAUA	794	UUUAUCU U CACCUUU
582	UGCUCAU C UAUACAC	795	UUAUCUU C ACCUUUC
584	CUCAUCU A UACACGG	800	UUCACCU U UCUCUAU
586	CAUCUAU A CACGGUU	801	UCACCUU U CUCUAUA
593	ACACGGU U ACCCAGA	802	CACCUUU C UCUAUAG
594	CACGGUU A CCCAGAA	804	CCUUUCU C UAUAGAG
605	AGAACCU A AGAAGAU	806	UUUCUCU A UAGAGCU
619	UGAGUGU U UUGCUIA	808	UCUCUAU A GAGCUUG
620	GAGUGUU U UGCUAAG	814	UAGAGCU U GAGGACC
621	AGUGUUU U GCUAAGA	824	GGACCCU C AGCCUCC
625	UUUUGCU A AGAACCA	830	UCAGCCU C CCCCAGA
638	CAAGAAU U CAACUAU	844	ACCACAU U CCTUGGA
639	AAGAAUU C AACUAUC	845	CCACAUU C CUUGGAU
644	UUCAACU A UCGAGUA	848	CAUUCUU U GGAUUAC
646	CAACUAU C GAGUAUG	853	CUUGGAU U ACAGCUG
651	AUCGAGU A UGAUGGU	854	UUGGAUU A CAGCUGU
659	UGAUGGU A UUAUGCA	862	CAGCUGU A CUUCCAA
661	AUGGUUU U AUGCAGA	865	CUGUACU U CCAACAG
662	UGGUUUU A UGCAGAA	866	UGUACUU C CAACAGU
672	CAGAAAU C UCAAGAU	874	CAACAGU U AUUAUAU
674	GAAAUUC C AAGAUAA	875	AACAGUU A UUAUAUG
680	UCAAGAU A AUGUCAC	877	CAGUUUU U AUAUGUG
685	AUAAGUU C ACAGAAC	878	AGUUUUU A UAUGUGU
696	GAACUGU A CGACGUU	880	UUAUUUU A UGUGUGA
703	ACGACGU U UCCAUCA	892	UGAUGGU U UUCUGUC
704	CGACGUU U CCAUCAG	893	GAUGGUU U UCUGUCU
705	GACGUUU C CAUCAGC	894	AUGGUUU U CUGUCUA
709	UUUCCAU C AGCUUGU	895	UGGUUUU C UGUCUAA
714	AUCAGCU U GUCUGUU	899	UUUCUGU C UAAUUCU
717	AGCUUGU C UGUUUCA	901	UCUGUCU A AUUCUAU

904 GUCUAAU U CUAUGGA
905 UCUAUUU C UAUGGAA
907 UAAUUCU A UGGAAAU
935 GCGGCCU C GCAACUC
942 CGCAACU C UUAUAAA
944 CAACUCU U AUAAAUG
945 AACUCUU A UAAAUGU
947 CUCUUUU A AAUGUGG
1009 AAAAAAU C CAUAUAC
1013 AAUCCAU A UACCUGA
1015 UCCAUUU A CCUGAAA
1026 GAAAGAU C UGAUGAA
1045 AGCGUGU U UUUAAAA
1046 GCGUGUU U UUUAAAG
1047 CGUGUUU U UAAAAGU
1048 GUGUUUU U AAAAGUU
1049 UGUUUUU A AAAGUUC
1055 UAAAAGU U CGAAGAC
1056 AAAAGUU C GAAGACA
1065 AAGACAU C UUCAUGC
1067 GACAUCU U CAUGCGA
1068 ACAUCUU C AUGCGAC
1085 AAGUGAU A CAUGUUU
1091 UACAUGU U UUUAAUU
1092 ACAUGUU U UUAUUUA
1093 CAUGUUU U UAAUUUA
1094 AUGUUUU U AAUUAAA
1095 UGUUUUU A AUUAAAG
1098 UUUUAAU U AAAGAGU
1099 UUUAAUU A AAGAGUA

Table BVII: Human B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
16	AGAAGCA CUGAUGAGGCCGAAAGGCCGAA AGCUUUC
17	GAGAAGC CUGAUGAGGCCGAAAGGCCGAA AAGCUUU
21	AGCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCAAAG
22	CAGCAGA CUGAUGAGGCCGAAAGGCCGAA AAGCAAA
24	AGCAGCA CUGAUGAGGCCGAAAGGCCGAA AGAAGCA
34	UCCUGU CUGAUGAGGCCGAAAGGCCGAA ACAGCAG
44	UCUGUGC CUGAUGAGGCCGAAAGGCCGAA AGUCCU
70	UGGAAAU CUGAUGAGGCCGAAAGGCCGAA ACCCCAC
73	AUCUGGA CUGAUGAGGCCGAAAGGCCGAA AUGACCC
74	UAUCUGG CUGAUGAGGCCGAAAGGCCGAA AAUGACC
75	AUAUCUG CUGAUGAGGCCGAAAGGCCGAA AAAUGAC
81	GACCUAA CUGAUGAGGCCGAAAGGCCGAA AUCUGGA
83	GUGACCU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
84	UGUGACC CUGAUGAGGCCGAAAGGCCGAA AAUAUCU
88	CUGCUGU CUGAUGAGGCCGAAAGGCCGAA ACCUAAU
113	CACUGGG CUGAUGAGGCCGAAAGGCCGAA AUCCAUU
125	AGUCCCA CUGAUGAGGCCGAAAGGCCGAA AGUGCAC
137	AGAAUGU CUGAUGAGGCCGAAAGGCCGAA ACUCAGU
142	CAAAGAG CUGAUGAGGCCGAAAGGCCGAA AUGUUAC
143	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAUGUUA
145	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AGAAUGU
147	CAUCACA CUGAUGAGGCCGAAAGGCCGAA AGAGAAU
148	CCAUCAC CUGAUGAGGCCGAAAGGCCGAA AAGAGAA
159	GAGCAGG CUGAUGAGGCCGAAAGGCCGAA AGGCCAU
160	AGAGCAG CUGAUGAGGCCGAAAGGCCGAA AAGGCCA
166	CACCAGA CUGAUGAGGCCGAAAGGCCGAA AGCAGGA
168	AGCACCA CUGAUGAGGCCGAAAGGCCGAA AGAGCAG
179	UUCAGAG CUGAUGAGGCCGAAAGGCCGAA AGCAGCA
182	AUCUUCA CUGAUGAGGCCGAAAGGCCGAA AGGAGCA
190	AAGCUUG CUGAUGAGGCCGAAAGGCCGAA AUCUUCA
191	UAAGCUU CUGAUGAGGCCGAAAGGCCGAA AAUCUUC
197	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGA
198	AUUGAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
200	UCAUUGA CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
201	CUCAUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAGC
202	UCUCAUU CUGAUGAGGCCGAAAGGCCGAA AAAUAAG
231	GUUUGCA CUGAUGAGGCCGAAAGGCCGAA AUUGGCA
232	AGUUUGC CUGAUGAGGCCGAAAGGCCGAA AAUUGGC
240	GUUUUGA CUGAUGAGGCCGAAAGGCCGAA AGUUUGC
242	UGGUUUU CUGAUGAGGCCGAAAGGCCGAA AGAGUUU
265	AUACUAC CUGAUGAGGCCGAAAGGCCGAA AGCUCAC

268 AAAAUAC CUGAUGAGGCCGAAAGGCCGAA ACUAGCU
271 GCCAAAA CUGAUGAGGCCGAAAGGCCGAA ACUACUA
273 CUGCCAA CUGAUGAGGCCGAAAGGCCGAA AUACUAC
274 CCUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUACUA
275 UCCUGCC CUGAUGAGGCCGAAAGGCCGAA AAUACU
294 CAGAAC CUGAUGAGGCCGAAAGGCCGAA AGUUUUC
298 CAUUCAG CUGAUGAGGCCGAAAGGCCGAA ACCAAGU
299 UCAUUA CUGAUGAGGCCGAAAGGCCGAA AACCAAG
310 CUAAGUA CUGAUGAGGCCGAAAGGCCGAA ACCUCAU
312 GCCUAAG CUGAUGAGGCCGAAAGGCCGAA AUACCUC
315 UUUGCCU CUGAUGAGGCCGAAAGGCCGAA AGUAUAC
316 CUUUGCC CUGAUGAGGCCGAAAGGCCGAA AAGUAUA
330 ACUGUCA CUGAUGAGGCCGAAAGGCCGAA AUUUCUC
331 CACUGUC CUGAUGAGGCCGAAAGGCCGAA AAUUCU
340 UGGA AUG CUGAUGAGGCCGAAAGGCCGAA ACACUGU
341 UUGGAU CUGAUGAGGCCGAAAGGCCGAA AACACUG
344 UACUUG CUGAUGAGGCCGAAAGGCCGAA AUGAACA
345 AUACUUG CUGAUGAGGCCGAAAGGCCGAA AAUGAAC
351 GCCCAUA CUGAUGAGGCCGAAAGGCCGAA ACUUGGA
353 CGGCCA CUGAUGAGGCCGAAAGGCCGAA AUACUUG
368 GAAUCA CUGAUGAGGCCGAAAGGCCGAA ACUUGUG
369 CGAAUCA CUGAUGAGGCCGAAAGGCCGAA AACUUGU
370 CCGAUC CUGAUGAGGCCGAAAGGCCGAA AAACUUG
374 CUGUCCG CUGAUGAGGCCGAAAGGCCGAA AUCAAAA
375 ACUGUCC CUGAUGAGGCCGAAAGGCCGAA AAUCAA
383 AGGGUCC CUGAUGAGGCCGAAAGGCCGAA ACUGUCC
397 GAUUGUG CUGAUGAGGCCGAAAGGCCGAA AGUCUCA
398 AGAUUGU CUGAUGAGGCCGAAAGGCCGAA AAGUCUC
404 AUCUGAA CUGAUGAGGCCGAAAGGCCGAA AUUGUGA
406 UGAUCUG CUGAUGAGGCCGAAAGGCCGAA AGAUUGU
407 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AAGAUUG
412 UGUCCUU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA
426 UUGAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCUU
429 ACAUUGA CUGAUGAGGCCGAAAGGCCGAA ACAAGCC
431 AUACAUU CUGAUGAGGCCGAAAGGCCGAA AUACAAG
437 UGGAUGA CUGAUGAGGCCGAAAGGCCGAA ACAUUGA
439 GAUGGAU CUGAUGAGGCCGAAAGGCCGAA AUACAUU
442 UGUGAUG CUGAUGAGGCCGAAAGGCCGAA AUGAUAC
446 UUUUUGU CUGAUGAGGCCGAAAGGCCGAA AUGGAUG
469 GGAUGCG CUGAUGAGGCCGAAAGGCCGAA AUCAUUC
470 UGGAUGC CUGAUGAGGCCGAAAGGCCGAA AAUCAUU
475 UCUGGUG CUGAUGAGGCCGAAAGGCCGAA AUGCGAA
488 AGUUCAG CUGAUGAGGCCGAAAGGCCGAA AUUCAUC
489 CAGUUA CUGAUGAGGCCGAAAGGCCGAA AAUUCAU
498 AAGCACU CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
505 AGUUAGC CUGAUGAGGCCGAAAGGCCGAA AGCACTUG
509 CUGAAGU CUGAUGAGGCCGAAAGGCCGAA AGCAAGC
513 UUGACUG CUGAUGAGGCCGAAAGGCCGAA AGUUAGC
514 GUUGACU CUGAUGAGGCCGAAAGGCCGAA AAGUUAG

518 UCAGGUU CUGAUGAGGCCGAAAGGCCGAA ACUGAAG
529 UUGGUAC CUGAUGAGGCCGAAAGGCCGAA AUUUCAG
532 AAAUUGG CUGAUGAGGCCGAAAGGCCGAA ACUAUUU
538 UAUUAGA CUGAUGAGGCCGAAAGGCCGAA AUUGGUA
539 AUUUUAG CUGAUGAGGCCGAAAGGCCGAA AAUUGGU
540 UAUUUUA CUGAUGAGGCCGAAAGGCCGAA AAAUUGG
542 GUUAUUA CUGAUGAGGCCGAAAGGCCGAA AGAAAUU
545 UCUGUUA CUGAUGAGGCCGAAAGGCCGAA AUUAGAA
547 UUUCUGU CUGAUGAGGCCGAAAGGCCGAA AUUUUAG
561 AUUUUAG CUGAUGAGGCCGAAAGGCCGAA ACACAUU
565 UCAAAUU CUGAUGAGGCCGAAAGGCCGAA AUGUACA
569 CAGGUCA CUGAUGAGGCCGAAAGGCCGAA AUUUUAG
570 GCAGGUC CUGAUGAGGCCGAAAGGCCGAA AAUUUUA
579 UAUAGAU CUGAUGAGGCCGAAAGGCCGAA AGCAGGU
582 GUGUAUA CUGAUGAGGCCGAAAGGCCGAA AUGAGCA
584 CCGUGUA CUGAUGAGGCCGAAAGGCCGAA AGAUGAG
586 AACCGUG CUGAUGAGGCCGAAAGGCCGAA AUAGAUG
593 UCUGGGU CUGAUGAGGCCGAAAGGCCGAA ACCGUGU
594 UUCUGGG CUGAUGAGGCCGAAAGGCCGAA AACCGUG
605 AUCUUCU CUGAUGAGGCCGAAAGGCCGAA AGGUUCU
619 UUAGCAA CUGAUGAGGCCGAAAGGCCGAA ACACUCA
620 CUUAGCA CUGAUGAGGCCGAAAGGCCGAA AACACUC
621 UCUUAGC CUGAUGAGGCCGAAAGGCCGAA AAACACU
625 UGUUUCU CUGAUGAGGCCGAAAGGCCGAA AGCAAAA
638 AUAGUUG CUGAUGAGGCCGAAAGGCCGAA AUUCUUG
639 GAUAGUU CUGAUGAGGCCGAAAGGCCGAA AAUUCUU
644 UACUCGA CUGAUGAGGCCGAAAGGCCGAA AGUUGAA
646 CAUACUC CUGAUGAGGCCGAAAGGCCGAA AUAGUUG
651 ACCAUCA CUGAUGAGGCCGAAAGGCCGAA ACUCGAU
659 UGCAUAA CUGAUGAGGCCGAAAGGCCGAA ACCAUCA
661 UCUGCAU CUGAUGAGGCCGAAAGGCCGAA AUACCAU
662 UUCUGCA CUGAUGAGGCCGAAAGGCCGAA AAUACCA
672 AUCUUGA CUGAUGAGGCCGAAAGGCCGAA AUUUCUG
674 UUAUCUU CUGAUGAGGCCGAAAGGCCGAA AGAUUUC
680 GUGACAU CUGAUGAGGCCGAAAGGCCGAA AUCUUGA
685 GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUUA
696 AACGUCG CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
703 UGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACGUCGU
704 CUGAUGG CUGAUGAGGCCGAAAGGCCGAA AACGUCG
705 GCUGAUG CUGAUGAGGCCGAAAGGCCGAA AAACGUC
709 ACAAGCU CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
714 AACAGAC CUGAUGAGGCCGAAAGGCCGAA AGCTUGAU
717 UGAAACA CUGAUGAGGCCGAAAGGCCGAA ACAAGCU
721 GGAAUGA CUGAUGAGGCCGAAAGGCCGAA ACAGACA
722 GGGAAUG CUGAUGAGGCCGAAAGGCCGAA AACAGAC
723 AGGGAAU CUGAUGAGGCCGAAAGGCCGAA AAACAGA
726 AUCAGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAC
727 CAUCAGG CUGAUGAGGCCGAAAGGCCGAA AAUGAAA
736 UGCUCGU CUGAUGAGGCCGAAAGGCCGAA ACAUCAG

737 UUGCUCG CUGAUGAGGCCGAAAGGCCGAA AACAUCA
746 AUGGUCA CUGAUGAGGCCGAAAGGCCGAA AUUGCUC
754 UACAGAA CUGAUGAGGCCGAAAGGCCGAA AUGGUCA
756 AAUACAG CUGAUGAGGCCGAAAGGCCGAA AGAUGGU
757 GAAUACA CUGAUGAGGCCGAAAGGCCGAA AAGAUGG
761 UCCAGAA CUGAUGAGGCCGAAAGGCCGAA ACAGAAG
763 UUUCCAG CUGAUGAGGCCGAAAGGCCGAA AUACAGA
764 GUUCCA CUGAUGAGGCCGAAAGGCCGAA AAUACAG
787 AAGAUAA CUGAUGAGGCCGAAAGGCCGAA AGCCCGC
788 GAAGAUU CUGAUGAGGCCGAAAGGCCGAA AAGCCGC
789 UGAAGAU CUGAUGAGGCCGAAAGGCCGAA AAAGCCG
790 GUGAAGA CUGAUGAGGCCGAAAGGCCGAA AAAAGCC
792 AGGUGAA CUGAUGAGGCCGAAAGGCCGAA AUAAAAG
794 AAAGGUG CUGAUGAGGCCGAAAGGCCGAA AGAUAAA
795 GAAAGGU CUGAUGAGGCCGAAAGGCCGAA AAGAUAA
800 AUAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGUGAA
801 UAUAGAG CUGAUGAGGCCGAAAGGCCGAA AAGGUGA
802 CUUAGA CUGAUGAGGCCGAAAGGCCGAA AAAGGUG
804 CUCUAUA CUGAUGAGGCCGAAAGGCCGAA AGAAAGG
806 AGCUCUA CUGAUGAGGCCGAAAGGCCGAA AGAGAAA
808 CAAGCUC CUGAUGAGGCCGAAAGGCCGAA AUAGAGA
814 GGUCUC CUGAUGAGGCCGAAAGGCCGAA AGCUCUA
824 GGAGGCU CUGAUGAGGCCGAAAGGCCGAA AGGGUCC
830 UCUGGGG CUGAUGAGGCCGAAAGGCCGAA AGGCUGA
844 UCCAAGG CUGAUGAGGCCGAAAGGCCGAA AUGUGGU
845 AUCCAAG CUGAUGAGGCCGAAAGGCCGAA AAUGUGG
848 GUAAUCC CUGAUGAGGCCGAAAGGCCGAA AGGAAUG
853 CAGCUGU CUGAUGAGGCCGAAAGGCCGAA AUCCAAG
854 ACAGCUG CUGAUGAGGCCGAAAGGCCGAA AAUCCAA
862 UUGGAAG CUGAUGAGGCCGAAAGGCCGAA ACAGCUG
865 CUGUUGG CUGAUGAGGCCGAAAGGCCGAA AGUACAG
866 ACUGUUG CUGAUGAGGCCGAAAGGCCGAA AAGUACA
874 AUUAAAU CUGAUGAGGCCGAAAGGCCGAA ACUGUUG
875 CAUAUAA CUGAUGAGGCCGAAAGGCCGAA AACUGUU
877 CACAUAU CUGAUGAGGCCGAAAGGCCGAA AUACUG
878 ACACAUU CUGAUGAGGCCGAAAGGCCGAA AAUAACU
880 UCACACA CUGAUGAGGCCGAAAGGCCGAA AUAAUAA
892 GACAGAA CUGAUGAGGCCGAAAGGCCGAA ACCAUCA
893 AGACAGA CUGAUGAGGCCGAAAGGCCGAA AAACCAU
894 UAGACAG CUGAUGAGGCCGAAAGGCCGAA AAACCAU
895 UUAGACA CUGAUGAGGCCGAAAGGCCGAA AAAACCA
899 AGAAUUA CUGAUGAGGCCGAAAGGCCGAA ACAGAAA
901 AUAGAAU CUGAUGAGGCCGAAAGGCCGAA AGACAGA
904 UCCAUAG CUGAUGAGGCCGAAAGGCCGAA AUUAGAC
905 UUCCAUA CUGAUGAGGCCGAAAGGCCGAA AAUUAGA
907 AUUCCA CUGAUGAGGCCGAAAGGCCGAA AGAAUUA
935 GAGUUGC CUGAUGAGGCCGAAAGGCCGAA AGGCCGC
942 UUUAAUA CUGAUGAGGCCGAAAGGCCGAA AGUUGCG
944 CAUUUAU CUGAUGAGGCCGAAAGGCCGAA AGAGUUG

945	ACAUUUA	CUGAUGAGGCCGAAAGGCCGAA	AAGAGUU
947	CCACAUU	CUGAUGAGGCCGAAAGGCCGAA	AUAAGAG
1009	GUAUAUG	CUGAUGAGGCCGAAAGGCCGAA	AUUTUUU
1013	UCAGGUA	CUGAUGAGGCCGAAAGGCCGAA	AUGGAUU
1015	UUUCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUAUGGA
1026	UUCAUCA	CUGAUGAGGCCGAAAGGCCGAA	AUCUUUC
1045	UUUUAUA	CUGAUGAGGCCGAAAGGCCGAA	ACACGCU
1046	CUUUUAA	CUGAUGAGGCCGAAAGGCCGAA	AACACGC
1047	ACUUUUA	CUGAUGAGGCCGAAAGGCCGAA	AAACACG
1048	AACUUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAACAC
1049	GAACUUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAACA
1055	GUCUUCG	CUGAUGAGGCCGAAAGGCCGAA	ACUUUUA
1056	UGUCUUC	CUGAUGAGGCCGAAAGGCCGAA	AACUUUU
1065	GCAUGAA	CUGAUGAGGCCGAAAGGCCGAA	AUGUCUU
1067	UCGCAUG	CUGAUGAGGCCGAAAGGCCGAA	AGAUGUC
1068	GUCGCAU	CUGAUGAGGCCGAAAGGCCGAA	AAGAUGU
1085	AAACAUG	CUGAUGAGGCCGAAAGGCCGAA	AUCACUU
1091	AAUUAUA	CUGAUGAGGCCGAAAGGCCGAA	ACAUGUA
1092	UAAUUAA	CUGAUGAGGCCGAAAGGCCGAA	AACAUGU
1093	UUAAUUA	CUGAUGAGGCCGAAAGGCCGAA	AAACAUG
1094	UUUAAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAACAU
1095	CUUUAUU	CUGAUGAGGCCGAAAGGCCGAA	AAAAACA
1098	ACUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUAAAA
1099	UACUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUA

Table BVIII: Mouse B7-2 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
47	AcGGACU u GaACAac	194	cuUAuUU C aAUGGgA
47	aCggACU u gaAcAAC	208	acUGCaU a UUGCcG
66	CUccUgU a gAcGUGU	210	UGCaUaU C UGCcGug
66	CUCcUgU A gAcGUGu	223	UGCCcAU U UaCAAag
74	gAcGUGU u CcagAAc	223	UGCcCAU u UAcAaAg
83	CaGaACU U aCggaAG	224	GCCcAUU U aCAAagg
134	caAuCcU U aUCUUUG	225	ccCAUUU a CAAaggc
134	CaauccU U AUCUUug	225	CccaUUU a cAAAgGc
134	caAUCcU u AuCUUUG	242	AAaACAU a agCcUGa
134	CAaUccU U AUcUuUG	260	AGCUgGU A GUAUUUU
134	CAAuccU U AUcUuUG	260	aGCUgGU a gUAUuUU
135	aAuCcUU a UC UUUGU	263	UgGUAGU A UUUUGGC
135	aAuCcUU a UC UUUGu	263	UGgUaGU a UUuUGgC
135	AaUccUU A UcUuUGU	265	GUAGUAU U UUGGCAG
135	aAUccUU a UC UUUGU	265	guAGUAU u UuGGCaG
137	uCCUUaU C UUUGUGA	266	UAGUAUU U UGGCAGG
137	UccUUaU c UuUGUGA	266	uAGUaUU U UGgcAgG
137	UCCuUAU c uuUGUGA	266	UAgUauU u UGGcAgg
139	cUUAUCU U UGUGAca	267	AGUAUUU U GGCAGGA
140	UUaUCUU U GUGAcaG	267	AGUaUUU U GgcAgGA
140	UUaUcuU U guGACAG	286	cAAAagU U GGUUCUG
149	UGAcaGU c UUGCUgA	286	CAAaagU U GgUUCuG
151	AcAGucU U GCUgaUC	290	AgUUGGU U CUGuAcG
151	AcaGuCU U gCUGaUC	291	gUUGGUU C UGuAcGA
158	UgCUGAU c UCAGaUg	295	GUUCuGU a CgAGcAc
158	UgCUGAU C UCAGaUG	304	GAGcacU A uUUGGC
158	UGcUgAU c uCAGaUg	307	cacUAUU u GGgCACA
158	UgCugAU c UCAGaUg	323	AGAAAcU U GAuAGUG
160	CUGaUCU C aGaUGCU	343	gCCAAGU A ccUGGGC
160	cUGaUcU c AgAuGcU	343	gCCAagU a CCUGGGc
170	AUGcuGU u UcCgUgG	361	ACgAGcU U UGAcagG
171	UGCUGuU u CcgUGgA	381	cUGgACU c UacGACU
172	gCUgUuU C cgUgGAG	383	GgACUcU A CGACuUc
189	GcaaGcU u AUUUCaA	383	GGACuCU a cGaCUuC
189	gCAAGCU U AUUUCAA	389	uAcGacU u CaCAaUG
189	GCaaGCU u AuUUCaA	389	UacGACU U CACAAUG
190	CAAGCUU A UUUCAAU	390	acGACUU C ACAAUgU
190	CaAgcUU a uUUCaAU	390	ACgAcUU c acAAUgU
192	AGCUUAU U UCAAUgG	398	ACAAUGU U CAgauCA
192	aGCUUaU u UCAAUgG	398	ACAAUGU U CAGAUCA
193	GCUUAUU U CAAUGgG	398	ACaAuGU U cagAUCA
193	GcuUAuU U CaAUGGg	399	CAaUGUU C AgauCAA
194	CUUAUUU C AAUGgGA	399	CAAUgUU C AGAUCAA

399	CaAuGUU c agAUCaa	658	CAGAUAU c AcaagAu
399	caAUGUU c aGAuCAA	658	CagauAU C ACAAgAu
399	CAaUguU c aGAUCaa	658	CAGauAU C aCAAGAU
399	cAAuGuU C aGAUCAA	658	CaGAUaU c ACaAGau
399	CAaugUU c agAUCAA	666	aCAAGAU A AUGUCAC
404	UUCAGAU C AAGGACA	666	ACAagaU a AUGucAC
404	UucAGaU c aAGGACa	671	AUaAuGU C ACAGaAc
418	aUGgGCU c GUAuGAU	671	aUAAUgU c ACAGAAC
418	AuGGGCU c GUAUgAu	671	AUAAUGU C ACAGAAC
418	AUggGCU c GUAUGaU	682	gAACUgU u cAGUAUc
421	gGCUCgU a UGAuugU	683	aAcUGuU c aGuAUcU
421	ggCUCgU A UgAuUGU	683	AAcUGuU c agUaUcU
429	UgAuUGU u UuAUaCA	691	aguaUcU C CAaCAGC
429	UGAUuGU u UUAUaCA	691	agUAUCU c CAaCagc
431	AuUgUuU u AUAcAAa	691	agUAucU C CAACAGc
431	AUuGUuU U AUaCAaa	701	aCaGCcU c UcUCUuU
432	UuGUuUU A UaCAaaA	701	acagCCU c UCUCUuU
432	UuGUUUU a UacaaAA	703	AGCcUcU C UcUUUCA
432	uUGUUUU a uAcaAAA	703	aGCcUcU c UCuuuca
461	gAUcaAU u AUCCucC	707	UcUCUcU U UCAUucc
462	AucaAUU a uCcUCCA	707	UcUCUcU u UcAUUcc
464	CAauUaU c CUcCaAc	708	cUCUcUU U CAUuccC
467	uUAUCcU C CAaCagA	709	UCUcUUU C AUUCCCg
467	UUauCcU C CAaCAGA	709	UCUCUuU c auuCccG
467	UUaUccU c cAACAGA	709	UCUcUuU c AUUcccg
467	UuAuCCU C CaaCAGA	712	CUUucaU U CcCgGaU
490	GAACUGU C AGUGaUc	712	cuuUCAU U cCCgGAU
497	CAGUGaU c GCcAACU	712	CuUucaU u CcCGGaU
505	GCcAACU U CAGUgAA	712	cUUUCAU U CCCgGAU
506	CcAACUU C AGUgAAC	712	CUUucaU u ccCggaU
506	CCAaCUU C aGUgaaC	713	uuUCAUu c CCgGAUg
521	CUGAAAU A aaACugg	713	UUUCAUu C CCgGAUG
531	ACUGgcU c AgAaUgU	732	GuGgcAU a UGACCgU
539	agaaUGU A ACAGGaA	732	GuGgcAU A UGACCgU
550	GgAaAuU c uGGCAuA	740	UGACCgU u gUgUGUg
550	ggAAaUU C UggcAUa	749	UgUGUgU U CUGGAAA
557	cuggCAU A AAUUGA	749	uGuGUGU U cUggAAA
561	CAUAAAU U UGACCUG	750	gUGUGUU C UGGAAAC
562	AUAAAUU U GACCUGC	750	GuGUGUU c UggAAAc
576	CaCgUCU A agCAaGG	773	ugAAGaU U UcCUcCa
585	gCAaGGU c ACCCgaA	778	aUUUcCU c caAACCu
597	gaAACCU A AGAAGAU	788	AAcCUCU C AAuuuCA
607	AaGaUgU a uUuUCUg	798	UUUCaCU c aAGAGuU
611	UGUaUUU u cUgAuAa	805	CAagAGU U UccAUcu
625	AcuAAAU C AACUaau	805	CAagAGU U uccAUcU
630	UUCAACU A auGAGUA	806	AAgAGUU u ccAUcUc
630	UUCaAcU A AuGAGUA	811	UUUCCAU C ucCUcaa
637	AauGAGU A UGgUGaU	811	uUUCaU c UcCUcaA
656	uGCAGaU a UcAcAAg	813	uCCAUCU c CUcaAac

836 aGgAGAU U acAGCUU
836 aggaGAU U ACAGCUu
837 GgAGAUU a cAGCUUc
848 CUUCAGU u AcugUGg
860 UGGCCcU C CUcCUug
860 UggCCcU c CUCcuUg
878 ugCUGCU C AUCauUg
951 GCGGgaU a GuAACgC
974 AgaCuAU c aACCTGA
989 aGgaAcU U GaACCCc
1006 auUgCUU c aGCAAAa
1055 AAAGAGU u aaAAaUU
1056 AaGAgUU a aaAAuUG
1062 uAAAAAU u gcUuUgC
1092 CAgaGUU u CuCAGAA
1095 aGUUUcU c AgAaUUC
1101 UCAGAAU u caaAaAU
1101 ucAGAAU U CAaAaAU
1101 UcAgAaU U CaAaAaU
1111 aAaAUGU U cUcAgcU
1112 AaAUGUU c UcAgcUg
1128 UUGGAaU u cuACAGU
1128 UUGGAaU u CuaCaGU
1131 GAAuUCU a cAGuUgA
1131 GAauUCU a CAguuGA
1141 GuUGAAU a aUuAAag
1144 gaaUAAU U AAAGAac
1145 AAuAaUU a aAgACA

Table BIX: Mouse B7-2 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
47	GUUGUUC CUGAUGAGGCCGAAAGGCCGAA AGUCCGU
47	GUUGUUC CUGAUGAGGCCGAAAGGCCGAA AGUCCGU
66	ACACGUC CUGAUGAGGCCGAAAGGCCGAA ACAGGAG
66	ACACGUC CUGAUGAGGCCGAAAGGCCGAA ACAGGAG
74	GUUCUGG CUGAUGAGGCCGAAAGGCCGAA ACACGUC
83	CUUCCGU CUGAUGAGGCCGAAAGGCCGAA AGUUCUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
134	CAAAGAU CUGAUGAGGCCGAAAGGCCGAA AGGAUUG
135	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAUU
135	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAUU
135	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAUU
135	ACAAAGA CUGAUGAGGCCGAAAGGCCGAA AAGGAUU
137	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUAAGGA
137	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUAAGGA
137	UCACAAA CUGAUGAGGCCGAAAGGCCGAA AUAAGGA
139	UGUCACA CUGAUGAGGCCGAAAGGCCGAA AGAUAAG
140	CUGUCAC CUGAUGAGGCCGAAAGGCCGAA AAGAUAA
140	CUGUCAC CUGAUGAGGCCGAAAGGCCGAA AAGAUAA
149	UCAGCAA CUGAUGAGGCCGAAAGGCCGAA ACUGUCA
151	GAUCAGC CUGAUGAGGCCGAAAGGCCGAA AGACUGU
151	GAUCAGC CUGAUGAGGCCGAAAGGCCGAA AGACUGU
158	CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
158	CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
158	CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
158	CAUCUGA CUGAUGAGGCCGAAAGGCCGAA AUCAGCA
160	AGCAUCU CUGAUGAGGCCGAAAGGCCGAA AGAUCAG
160	AGCAUCU CUGAUGAGGCCGAAAGGCCGAA AGAUCAG
170	CCACGGA CUGAUGAGGCCGAAAGGCCGAA ACAGCAU
171	UCCACGG CUGAUGAGGCCGAAAGGCCGAA AACAGCA
172	CUCCACG CUGAUGAGGCCGAAAGGCCGAA AACAGC
189	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGC
189	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGC
189	UUGAAAU CUGAUGAGGCCGAAAGGCCGAA AGCUUGC
190	AUUGAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
190	AUUGAAA CUGAUGAGGCCGAAAGGCCGAA AAGCUUG
192	CCAUUGA CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
192	CCAUUGA CUGAUGAGGCCGAAAGGCCGAA AUAAGCU
193	CCCAUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAGC
193	CCCAUUG CUGAUGAGGCCGAAAGGCCGAA AAUAAGC
194	UCCCAUU CUGAUGAGGCCGAAAGGCCGAA AAUAAG

194 UCCCAU CUGAUGAGGCCGAAAGGCCGAA AAAUAAG
208 CGGCAGA CUGAUGAGGCCGAAAGGCCGAA AUGCAGU
210 CACGGCA CUGAUGAGGCCGAAAGGCCGAA AU AUGCA
223 CUUUGUA CUGAUGAGGCCGAAAGGCCGAA AUGGGCA
223 CUUUGUA CUGAUGAGGCCGAAAGGCCGAA AUGGGCA
224 CCUUGU CUGAUGAGGCCGAAAGGCCGAA AAUGGGC
225 GCCUUG CUGAUGAGGCCGAAAGGCCGAA AAAUGGG
225 GCCUUG CUGAUGAGGCCGAAAGGCCGAA AAAUGGG
242 UCAGGCU CUGAUGAGGCCGAAAGGCCGAA AUGUUUU
260 AAAAUAC CUGAUGAGGCCGAAAGGCCGAA ACCAGCU
260 AAAAUAC CUGAUGAGGCCGAAAGGCCGAA ACCAGCU
263 GCCAAA CUGAUGAGGCCGAAAGGCCGAA ACUACCA
263 GCCAAA CUGAUGAGGCCGAAAGGCCGAA ACUACCA
265 CUGCCAA CUGAUGAGGCCGAAAGGCCGAA AUACUAC
265 CUGCCAA CUGAUGAGGCCGAAAGGCCGAA AUACUAC
266 CCUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUACUA
266 CCUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUACUA
266 CCUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUACUA
267 UCCUGCC CUGAUGAGGCCGAAAGGCCGAA AAAUACU
267 UCCUGCC CUGAUGAGGCCGAAAGGCCGAA AAAUACU
286 CAGAAC CUGAUGAGGCCGAAAGGCCGAA ACUUUUG
286 CAGAAC CUGAUGAGGCCGAAAGGCCGAA ACUUUUG
290 CGUACAG CUGAUGAGGCCGAAAGGCCGAA ACCAACU
291 UCGUACA CUGAUGAGGCCGAAAGGCCGAA AACCAAC
295 GUGCUCG CUGAUGAGGCCGAAAGGCCGAA ACAGAAC
304 GCCCAA CUGAUGAGGCCGAAAGGCCGAA AGUGCUC
307 UGUGCCC CUGAUGAGGCCGAAAGGCCGAA AAUAGUG
323 CACUAUC CUGAUGAGGCCGAAAGGCCGAA AGUUUCU
343 GCCCAGG CUGAUGAGGCCGAAAGGCCGAA ACUUGGC
343 GCCCAGG CUGAUGAGGCCGAAAGGCCGAA ACUUGGC
361 CCUGUCA CUGAUGAGGCCGAAAGGCCGAA AGCUCGU
381 AGUCGUA CUGAUGAGGCCGAAAGGCCGAA AGUCCAG
383 GAAGUCG CUGAUGAGGCCGAAAGGCCGAA AGAGUCC
383 GAAGUCG CUGAUGAGGCCGAAAGGCCGAA AGAGUCC
389 CAUUGUG CUGAUGAGGCCGAAAGGCCGAA AGUCGUA
389 CAUUGUG CUGAUGAGGCCGAAAGGCCGAA AGUCGUA
390 ACAUUGU CUGAUGAGGCCGAAAGGCCGAA AAGUCGU
390 ACAUUGU CUGAUGAGGCCGAAAGGCCGAA AAGUCGU
398 UGAUCUG CUGAUGAGGCCGAAAGGCCGAA ACAUUGU
398 UGAUCUG CUGAUGAGGCCGAAAGGCCGAA ACAUUGU
398 UGAUCUG CUGAUGAGGCCGAAAGGCCGAA ACAUUGU
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
399 UUGAUCU CUGAUGAGGCCGAAAGGCCGAA AACAUUG
404 UGUCCU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA

404 UGUCCUU CUGAUGAGGCCGAAAGGCCGAA AUCUGAA
418 AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU
418 AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU
418 AUCAUAC CUGAUGAGGCCGAAAGGCCGAA AGCCCAU
421 ACAAUCA CUGAUGAGGCCGAAAGGCCGAA ACGAGCC
421 ACAAUCA CUGAUGAGGCCGAAAGGCCGAA ACGAGCC
429 UGUUAUA CUGAUGAGGCCGAAAGGCCGAA ACAAUCA
429 UGUUAUA CUGAUGAGGCCGAAAGGCCGAA ACAAUCA
431 UUUGUAU CUGAUGAGGCCGAAAGGCCGAA AAACAAU
431 UUUGUAU CUGAUGAGGCCGAAAGGCCGAA AAACAAU
432 UUUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA
432 UUUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA
432 UUUUGUA CUGAUGAGGCCGAAAGGCCGAA AAAACAA
461 GGAGGAU CUGAUGAGGCCGAAAGGCCGAA AUUGAUC
462 UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AAUUGAU
464 GUUGGAG CUGAUGAGGCCGAAAGGCCGAA AUAUUG
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA
467 UCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGGAUAA
490 GAUCACU CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
497 AGUUGGC CUGAUGAGGCCGAAAGGCCGAA AUCACUG
505 UUCACUG CUGAUGAGGCCGAAAGGCCGAA AGUUGGC
506 GUUCACU CUGAUGAGGCCGAAAGGCCGAA AAGUUGG
506 GUUCACU CUGAUGAGGCCGAAAGGCCGAA AAGUUGG
521 CCAGUUU CUGAUGAGGCCGAAAGGCCGAA AUUUCAG
531 ACAUUCU CUGAUGAGGCCGAAAGGCCGAA AGCCAGU
539 UUCCTUG CUGAUGAGGCCGAAAGGCCGAA ACAUUCU
550 UAUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUUCC
550 UAUGCCA CUGAUGAGGCCGAAAGGCCGAA AAUUCC
557 UCAAAUU CUGAUGAGGCCGAAAGGCCGAA AUGCCAG
561 CAGGUCA CUGAUGAGGCCGAAAGGCCGAA AUUUAUG
562 GCAGGUC CUGAUGAGGCCGAAAGGCCGAA AAUUAU
576 CCUUGCU CUGAUGAGGCCGAAAGGCCGAA AGACGUG
585 UUCGGGU CUGAUGAGGCCGAAAGGCCGAA ACCUUGC
597 AUCUUCU CUGAUGAGGCCGAAAGGCCGAA AGGUUUC
607 CAGAAAA CUGAUGAGGCCGAAAGGCCGAA ACAUCUU
611 UUAUCAG CUGAUGAGGCCGAAAGGCCGAA AAUACA
625 AUUAGUU CUGAUGAGGCCGAAAGGCCGAA AAUAGU
630 UACUCAU CUGAUGAGGCCGAAAGGCCGAA AGUUGAA
630 UACUCAU CUGAUGAGGCCGAAAGGCCGAA AGUUGAA
637 AUCACCA CUGAUGAGGCCGAAAGGCCGAA ACUCAUU
656 CUUGUGA CUGAUGAGGCCGAAAGGCCGAA AUCUGCA
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
658 AUCUUGU CUGAUGAGGCCGAAAGGCCGAA AUAUCUG
666 GUGACAU CUGAUGAGGCCGAAAGGCCGAA AUCUUGU
666 GUGACAU CUGAUGAGGCCGAAAGGCCGAA AUCUUGU

671 GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUUAU
671 GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUUAU
671 GUUCUGU CUGAUGAGGCCGAAAGGCCGAA ACAUUUAU
682 GAUACUG CUGAUGAGGCCGAAAGGCCGAA ACAGUUC
683 AGAUACU CUGAUGAGGCCGAAAGGCCGAA AACAGUU
683 AGAUACU CUGAUGAGGCCGAAAGGCCGAA AACAGUU
691 GCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGAUACU
691 GCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGAUACU
691 GCUGUUG CUGAUGAGGCCGAAAGGCCGAA AGAUACU
701 AAAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGCUGU
701 AAAGAGA CUGAUGAGGCCGAAAGGCCGAA AGGCUGU
703 UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
703 UGAAAGA CUGAUGAGGCCGAAAGGCCGAA AGAGGCU
707 GGAAUGA CUGAUGAGGCCGAAAGGCCGAA AGAGAGA
707 GGAAUGA CUGAUGAGGCCGAAAGGCCGAA AGAGAGA
708 GGGAAUG CUGAUGAGGCCGAAAGGCCGAA AAGAGAG
709 CGGGAAU CUGAUGAGGCCGAAAGGCCGAA AAAGAGA
709 CGGGAAU CUGAUGAGGCCGAAAGGCCGAA AAAGAGA
709 CGGGAAU CUGAUGAGGCCGAAAGGCCGAA AAAGAGA
712 AUCCGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
712 AUCCGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
712 AUCCGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
712 AUCCGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
712 AUCCGGG CUGAUGAGGCCGAAAGGCCGAA AUGAAAG
713 CAUCCGG CUGAUGAGGCCGAAAGGCCGAA AAUGAAA
713 CAUCCGG CUGAUGAGGCCGAAAGGCCGAA AAUGAAA
732 ACGGUCA CUGAUGAGGCCGAAAGGCCGAA AUGCCAC
732 ACGGUCA CUGAUGAGGCCGAAAGGCCGAA AUGCCAC
740 CACACAC CUGAUGAGGCCGAAAGGCCGAA ACGGUCA
749 UUUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACACA
749 UUUCCAG CUGAUGAGGCCGAAAGGCCGAA ACACACA
750 GUUUCCA CUGAUGAGGCCGAAAGGCCGAA AACACAC
750 GUUUCCA CUGAUGAGGCCGAAAGGCCGAA AACACAC
773 UGGAGGA CUGAUGAGGCCGAAAGGCCGAA AUCUUCA
778 AGGUUUG CUGAUGAGGCCGAAAGGCCGAA AGGAAAU
788 UGAAAUU CUGAUGAGGCCGAAAGGCCGAA AGAGGUU
798 AACTUUU CUGAUGAGGCCGAAAGGCCGAA AGUGAAA
805 AGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACTUUUG
805 AGAUGGA CUGAUGAGGCCGAAAGGCCGAA ACTUUUG
806 GAGAUGG CUGAUGAGGCCGAAAGGCCGAA AACTUUU
811 UUGAGGA CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
811 UUGAGGA CUGAUGAGGCCGAAAGGCCGAA AUGGAAA
813 GUUUGAG CUGAUGAGGCCGAAAGGCCGAA AGAUGGA
836 AAGCUGU CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
836 AAGCUGU CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
837 GAAGCUG CUGAUGAGGCCGAAAGGCCGAA AAUCUCC
848 CCACAGU CUGAUGAGGCCGAAAGGCCGAA ACUGAAG
860 CAAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGGCCA
860 CAAGGAG CUGAUGAGGCCGAAAGGCCGAA AGGGCCA

878	CAAUGAU	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCA
951	GCGUUAC	CUGAUGAGGCCGAAAGGCCGAA	AUCCCGC
974	UCAGGUU	CUGAUGAGGCCGAAAGGCCGAA	AUAGUCU
989	GGGGUUC	CUGAUGAGGCCGAAAGGCCGAA	AGUUCU
1006	UUUUGCU	CUGAUGAGGCCGAAAGGCCGAA	AAGCAAU
1055	AAUUUUU	CUGAUGAGGCCGAAAGGCCGAA	ACUCUUU
1056	CAAUUUU	CUGAUGAGGCCGAAAGGCCGAA	AACUCUU
1062	GCAAAGC	CUGAUGAGGCCGAAAGGCCGAA	AUUUUUA
1092	UUCUGAG	CUGAUGAGGCCGAAAGGCCGAA	AACUCUG
1095	GAAUUCU	CUGAUGAGGCCGAAAGGCCGAA	AGAAACU
1101	AUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
1101	AUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
1101	AUUUUUG	CUGAUGAGGCCGAAAGGCCGAA	AUUCUGA
1111	AGCUGAG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUUU
1112	CAGCUGA	CUGAUGAGGCCGAAAGGCCGAA	AACAUUU
1128	ACUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAA
1128	ACUGUAG	CUGAUGAGGCCGAAAGGCCGAA	AUUCCAA
1131	UCAACUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUC
1131	UCAACUG	CUGAUGAGGCCGAAAGGCCGAA	AGAAUUC
1141	CUUUAAU	CUGAUGAGGCCGAAAGGCCGAA	AUUC AAC
1144	GUUCUUU	CUGAUGAGGCCGAAAGGCCGAA	AUUAUUC
1145	UGUUCUU	CUGAUGAGGCCGAAAGGCCGAA	AAUUAUU

Table BX: Human CD40 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
9	CCUCGCU C GGGCGCC	440	UUGGGGU C AAGCAGA
24	CAGUGGU C CUGCCGC	449	AGCAGAU U GCUACAG
37	GCCUGGU C UCACCUC	453	GAUUGCU A CAGGGGU
39	CUGGUCU C ACCUOGC	461	CAGGGGU U UCUGAUA
44	CUCACCU C GCCAUGG	462	AGGGGUU U CUGAUAC
53	CCAUGGU U CGUCUGC	463	GGGGUUU C UGAUACC
54	CAUGGUU C GUCUGCC	468	UUCUGAU A CCAUCUG
57	GGUUCGU C UGCCUCU	473	AUACCAU C UGCGAGC
63	UCUGCCU C UGCAGUG	491	GCCCAGU C GGCUCUC
74	AGUGCGU C CUCUGGG	496	GUCGGCU U CUUCUCC
77	GCGUCCU C UGGGGCU	497	UCGGCUU C UUCUCCA
88	GGCUGCU U GCUGACC	499	GGCUUCU U CUCCAUA
101	CCGCUGU C CAUCCAG	500	GCUUCTU C UCCAUG
105	UGUCCAU C CAGAAC	502	UUCUUCU C CAAUGUG
139	AAACAGU A CCTAAUA	511	AAUGUGU C AUCUGCU
143	AGUACCU A AUAACA	514	GUGUCAU C UGCUUUC
146	ACCUAAU A AACAGUC	519	AUCUGCU U UCGAAAA
153	AAACAGU C AGUGCUG	520	UCUGCUU U CGAAAAA
162	GUGCGU U CUUUGUG	521	CUGCUUU C GAAAAAU
163	UGCUGUU C UUUGUGC	531	AAAAUGU C ACCCUUG
165	CUGUUCU U UGUGCCA	537	UCACCCU U GGACAAG
166	UGUUCUU U GUGCCAG	566	ACCUGGU U GUGCAAC
208	ACAGAGU U CACUGAA	599	CUGAUGU U GUCUGUG
209	CAGAGUU C ACUGAAA	602	AUGUUGU C UGUGGUC
227	AAUGCCU U CCUUGCG	609	CUGUGGU C CCCAGGA
228	AUGCCUU C CUUGCGG	618	CCAGGAU C GGCUGAG
231	CCUUCUU U GCGGUGA	641	UGGUGAU C CCAUCA
247	AGCGAAU U CCUAGAC	647	UCCCCAU C AUCUUCG
248	GCGAAUU C CUAGACA	650	CCAUCAU C UUCGGGA
251	AAUUCUU A GACACCU	652	AUCAUCU U CGGGAUC
292	CACAAAU A CUGCGAC	653	UCAUCUU C GGGAUCC
308	CCAACCU A GGGCUUC	659	UCGGGAU C CUGUUUG
314	UAGGGCU U CGGGUCC	664	AUCCUGU U UGCCAUC
315	AGGGCUU C GGGUCCA	665	UCCUGUU U GCCAUCC
320	UUCGGGU C CAGCAGA	671	UUGCCAU C CUCUUGG
337	GGCACTU C AGAAACA	674	CCAUCCU C UUGGUGC
353	ACACCAU C UGCACCU	676	AUCCUCU U GGUGCUG
381	GCACUGU A CGAGUGA	686	UGCUGGU C UUAUCA
407	GCUGUGU C CUGCACC	688	CUGGUCU U UAUAACA
418	CACCGCU C AUGCUCG	689	UGGUCUU U AUCAAAA
424	UCAUGCU C GCCCGGC	690	GGUCUUU A UCAAAAA
433	CCCGGCU U UGGGGUC	692	UCUUUAU C AAAAAAG
434	CCGGCUU U GGGGUCA	720	AACCAAU A AGGCCCC

755 AGGAGAU C AAUUUUC
759 GAUCAAU U UUCCCGA
760 AUCAAUU U UCCCGAC
761 UCAAUUU U CCCGACG
762 CAAUUUU C CCGACGA
771 CGACGAU C UUCCUGG
773 ACGAUCU U CCUGGCU
774 CGAUCUU C CUGGCUC
781 CCUGGCU C CAACACU
795 UGCUGCU C CAGUGCA
810 GGAGACU U UACAUGG
811 GAGACUU U ACAUGGA
812 AGACUUU A CAUGGAU
830 AACCAGU C ACCCAGG
855 AGAGAGU C GCAUCUC
860 GUCCGAU C UCAGUGC
862 CGCAUCU C AGUGCAG
927 AGGCAGU U GGCCAGA
981 GGGAGCU A UGCCCAG
990 GCCCAGU C AGUGCCA

Table BXL: Human CD40 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequences
9	GGCGCCC CUGAUGAGGCCGAAAGGCCGAA AGCGAGG
24	GCGGCAG CUGAUGAGGCCGAAAGGCCGAA ACCACUG
37	GAGGUGA CUGAUGAGGCCGAAAGGCCGAA ACCAGGC
39	GCGAGGU CUGAUGAGGCCGAAAGGCCGAA AGACCAG
44	CCAUGGC CUGAUGAGGCCGAAAGGCCGAA AGGUGAG
53	GCAGACG CUGAUGAGGCCGAAAGGCCGAA ACCAUGG
54	GGCAGAC CUGAUGAGGCCGAAAGGCCGAA AACCAUG
57	AGAGGCA CUGAUGAGGCCGAAAGGCCGAA ACGAACC
63	CACUGCA CUGAUGAGGCCGAAAGGCCGAA AGGCAGA
74	CCCAGAG CUGAUGAGGCCGAAAGGCCGAA ACGCACU
77	AGCCCCA CUGAUGAGGCCGAAAGGCCGAA AGGACGC
88	GGUCAGC CUGAUGAGGCCGAAAGGCCGAA AGCAGCC
101	CUGGAUG CUGAUGAGGCCGAAAGGCCGAA ACAGCGG
105	GGUUCUG CUGAUGAGGCCGAAAGGCCGAA AUGGACA
139	UAUUAGG CUGAUGAGGCCGAAAGGCCGAA ACUGUUU
143	UGUUUUAU CUGAUGAGGCCGAAAGGCCGAA AGGUACU
146	GACUGUU CUGAUGAGGCCGAAAGGCCGAA AUUAGGU
153	CAGCACU CUGAUGAGGCCGAAAGGCCGAA ACUGUUU
162	CACAAAG CUGAUGAGGCCGAAAGGCCGAA ACAGCAC
163	GCACAAA CUGAUGAGGCCGAAAGGCCGAA AACAGCA
165	UGGCACA CUGAUGAGGCCGAAAGGCCGAA AGAACAG
166	CUGGCAC CUGAUGAGGCCGAAAGGCCGAA AAGAACA
208	UUCAGUG CUGAUGAGGCCGAAAGGCCGAA ACUCUGU
209	UUUCAGU CUGAUGAGGCCGAAAGGCCGAA AACUCUG
227	CGCAAGG CUGAUGAGGCCGAAAGGCCGAA AGGCAUU
228	CCGCAAG CUGAUGAGGCCGAAAGGCCGAA AAGGCAU
231	UCACCGC CUGAUGAGGCCGAAAGGCCGAA AGGAAGG
247	GUCUAGG CUGAUGAGGCCGAAAGGCCGAA AUUCGCU
248	UGUCUAG CUGAUGAGGCCGAAAGGCCGAA AAUUCGC
251	AGGUGUC CUGAUGAGGCCGAAAGGCCGAA AGGAAUU
292	GUCGCAG CUGAUGAGGCCGAAAGGCCGAA AUUUGUG
308	GAAGCCC CUGAUGAGGCCGAAAGGCCGAA AGGUUGG
314	GGACCCG CUGAUGAGGCCGAAAGGCCGAA AGCCCUA
315	UGGACCC CUGAUGAGGCCGAAAGGCCGAA AAGCCCU
320	UCUGCUG CUGAUGAGGCCGAAAGGCCGAA ACCCGAA
337	UGUUUCU CUGAUGAGGCCGAAAGGCCGAA AGGUGCC
353	AGGUGCA CUGAUGAGGCCGAAAGGCCGAA AUGGUGU
381	UCACUCG CUGAUGAGGCCGAAAGGCCGAA ACAGUGC
407	GGUGCAG CUGAUGAGGCCGAAAGGCCGAA ACACAGC
418	CGAGCAU CUGAUGAGGCCGAAAGGCCGAA AGCGGUG
424	GCCGGGC CUGAUGAGGCCGAAAGGCCGAA AGCAUGA
433	GACCCCA CUGAUGAGGCCGAAAGGCCGAA AGCCGGG
434	UGACCCC CUGAUGAGGCCGAAAGGCCGAA AAGCCGG

440 UCUGCUU CUGAUGAGGCCGAAAGGCCGAA ACCCCAA
449 CUGUAGC CUGAUGAGGCCGAAAGGCCGAA AUCUGCU
453 ACCCCUG CUGAUGAGGCCGAAAGGCCGAA AGCAAUC
461 UAUCAGA CUGAUGAGGCCGAAAGGCCGAA ACCCCUG
462 GUAUCAG CUGAUGAGGCCGAAAGGCCGAA AACCCCU
463 GGUAUCA CUGAUGAGGCCGAAAGGCCGAA AAACCCC
468 CAGAUGG CUGAUGAGGCCGAAAGGCCGAA AUCAGAA
473 GCUCGCA CUGAUGAGGCCGAAAGGCCGAA AUGGUUU
491 AGAAGCC CUGAUGAGGCCGAAAGGCCGAA ACUGGGC
496 GGAGAAG CUGAUGAGGCCGAAAGGCCGAA AGCCGAC
497 UGGAGAA CUGAUGAGGCCGAAAGGCCGAA AAGCCGA
499 AUUGGAG CUGAUGAGGCCGAAAGGCCGAA AGAAGCC
500 CAUUGGA CUGAUGAGGCCGAAAGGCCGAA AAGAAGC
502 CACAUUG CUGAUGAGGCCGAAAGGCCGAA AGAAGAA
511 AGCAGAU CUGAUGAGGCCGAAAGGCCGAA ACACAUU
514 GAAAGCA CUGAUGAGGCCGAAAGGCCGAA AUGACAC
519 UUUUCGA CUGAUGAGGCCGAAAGGCCGAA AGCAGAU
520 UUUUUCG CUGAUGAGGCCGAAAGGCCGAA AAGCAGA
521 AUUUUUC CUGAUGAGGCCGAAAGGCCGAA AAAGCAG
531 CAAGGGU CUGAUGAGGCCGAAAGGCCGAA ACAUUUU
537 CUUGUCC CUGAUGAGGCCGAAAGGCCGAA AGGGUGA
566 GUUGCAC CUGAUGAGGCCGAAAGGCCGAA ACCAGGU
599 CACAGAC CUGAUGAGGCCGAAAGGCCGAA ACAUCAG
602 GACCACA CUGAUGAGGCCGAAAGGCCGAA ACAACAU
609 UCCUGGG CUGAUGAGGCCGAAAGGCCGAA ACCACAG
618 CUCAGCC CUGAUGAGGCCGAAAGGCCGAA AUCCUGG
641 UGAUGGG CUGAUGAGGCCGAAAGGCCGAA AUCACCA
647 CGAAGAU CUGAUGAGGCCGAAAGGCCGAA AUGGGGA
650 UCCCGAA CUGAUGAGGCCGAAAGGCCGAA AUGAUGG
652 GAUCCCG CUGAUGAGGCCGAAAGGCCGAA AGAUGAU
653 GGAUCCC CUGAUGAGGCCGAAAGGCCGAA AAGAUGA
659 CAAACAG CUGAUGAGGCCGAAAGGCCGAA AUCCCGA
664 GAUGGCA CUGAUGAGGCCGAAAGGCCGAA ACAGGAU
665 GGAUGGC CUGAUGAGGCCGAAAGGCCGAA AACAGGA
671 CCAAGAG CUGAUGAGGCCGAAAGGCCGAA AUGGCAA
674 GCACCAA CUGAUGAGGCCGAAAGGCCGAA AGGAUGG
676 CAGCACC CUGAUGAGGCCGAAAGGCCGAA AGAGGAU
686 UGAUAAA CUGAUGAGGCCGAAAGGCCGAA ACCAGCA
688 UUUGAUA CUGAUGAGGCCGAAAGGCCGAA AGACCAG
689 UUUUGAU CUGAUGAGGCCGAAAGGCCGAA AAGACCA
690 UUUUUGA CUGAUGAGGCCGAAAGGCCGAA AAAGACC
692 CCUUUUU CUGAUGAGGCCGAAAGGCCGAA AUAAAGA
720 GGGGCTU CUGAUGAGGCCGAAAGGCCGAA AUUGGUU
755 GAAAAUU CUGAUGAGGCCGAAAGGCCGAA AUCUCCU
759 UCGGGAA CUGAUGAGGCCGAAAGGCCGAA AUUGAUC
760 GUCGGGA CUGAUGAGGCCGAAAGGCCGAA AAUUGAU
761 CGUCGGG CUGAUGAGGCCGAAAGGCCGAA AAAUUGA
762 UCGUCGG CUGAUGAGGCCGAAAGGCCGAA AAAAUUG
771 CCAGGA A CUGAUGAGGCCGAAAGGCCGAA AUCGUCC

773	AGCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AGAUCGU
774	GAGCCAG	CUGAUGAGGCCGAAAGGCCGAA	AAGAUCG
781	AGUGUUG	CUGAUGAGGCCGAAAGGCCGAA	AGCCAGG
795	UGCACUG	CUGAUGAGGCCGAAAGGCCGAA	AGCAGCA
810	CCAUGUA	CUGAUGAGGCCGAAAGGCCGAA	AGUCUCC
811	UCCAUGU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCUC
812	AUCCAUG	CUGAUGAGGCCGAAAGGCCGAA	AAAGUCU
830	CCUGGGU	CUGAUGAGGCCGAAAGGCCGAA	ACCGGUU
855	GAGAUGC	CUGAUGAGGCCGAAAGGCCGAA	ACUCUCU
860	GCACUGA	CUGAUGAGGCCGAAAGGCCGAA	AUGCGAC
862	CUGCACU	CUGAUGAGGCCGAAAGGCCGAA	AGAUGCG
927	UCUGGCC	CUGAUGAGGCCGAAAGGCCGAA	ACUGCCU
981	CUGGGCA	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCC
990	UGGCACU	CUGAUGAGGCCGAAAGGCCGAA	ACUGGGC

Table BXII: Mouse CD40 Hammerhead Ribozyme Target Sequences

nt. Position	HH Target Sequence	nt. Position	HH Target Sequence
18	GGUgucU u UGCCUCg	479	cAUCAcU U UUCgaaA
18	GGuguCU u UGCCucG	480	AUCacuU U UCGAAAA
24	UuUGCCU C gGCuGUG	481	UCacuUU U CGAAAAg
38	GCGcgCU a UGGGGCU	481	UCACuuU U cGaaAAG
62	CagcGGU c CaUCUag	492	AAAgUGU u AuCCcUG
62	CaGCgGU C CAUCuAG	560	CUaAUGU c aUCUGUG
66	gGUCCA U C uAGggCa	563	AUGUcaU C UGUGGUu
80	AGUGuGU u acgUGca	572	gUGGUuU a AagUCcC
80	AgUGUGU u AcgUGCa	572	GuGGUUU a aagUcCC
81	gUGugUU a CgUGCaG	577	UuAAaGU c CCgGAuG
100	AAACAGU A CTUccac	620	UGGgcAU C CuCAUCA
126	CUGUgaU U UGUGCCA	626	UCCuCAU C AcCaUuu
127	UGUgaUU U GUGCCAG	632	uCAcCAU u UUCGGGg
170	CAGcUcU u gaGAaGA	632	UcaCCA U uUCggGG
208	gGCGAAU U CUCAGcC	634	AcCAUuU U CGGGgUg
209	GCGAAUU C ucAGcCc	635	CCaUuuU c GgGGUGu
233	gGGAGAU u cgcUgUC	635	cCAUuuU C GGGgUgu
267	ACCCAAU c AAggGcu	635	CCAuuuU C ggGGUGu
267	AcCCAAU c AaGggCu	647	UGuUucU C UaUAUCA
275	aAGGGCU U CGGGUua	649	uUucUCU a UAUCAAA
275	AaGGGcU U CgGgUua	651	ucUCUaU A UCAAAAA
276	AGGGCUU C GGGUuaA	653	UCUaUAU C AAAAAGG
281	UUCGGGU u aAGaAGg	735	gGAaGAU u aUCCcGG
281	UUCGGGU u AAGaAGg	759	cGUGGUU C CAGUGCA
314	ACACugU C UGUACCU	794	AgCCuGU C ACaCAGG
354	caAgGaU u GCgaGGC	794	AGcCuGU c acaCAGg
386	cCugUaU c CCUGGCU	819	AGAGAGU C GCAUCUC
394	CCUGGCU u uGgaGuu	824	GUCGCAU C UCAGUGC
394	CCUGGCU U UGgaGUu	826	CGCAUCU C AGUGCAG
395	CuGGGCU U GGaGUuA	876	cCCUGGU C UgAaCcC
429	caCUGAU A CCgUCUG	913	GGCUGCU U GCUGACC
434	AUACCgU C UGucAuC	997	CUCAaCU u GCuuUuu
434	AUaCcGU c UGUCAUC	1003	uUGCUUU u uAAGgAU
441	CugUCAU C CcuGCcC	1003	uugCUUU u uAaGGAU
452	GCCCAGU C GGCUUUCU	1023	gaAAgCU c GGGCaUC
452	GCCCAGU C gGcuuCu	1048	CAGuGaU a UCUaccA
457	GUCGGCU U CUUCUCC	1052	gAUauCU a CCaaGuG
458	UCGGCUU C UUCUCCA	1081	CCAGagU u GuCUugc
460	GGCUUCU U CUCCAAU	1084	gAGUuGU C uUGCuGC
461	GCUUCUU C UCCAAUc	1086	gUugUCU U GcUGCgG
463	UUCUUCU C CAAUcaG	1097	gCgGcGU U CACUGuA
472	AAuCAGU C AucaCUu	1098	CgGcGUU C ACUGuAA
472	AAUcagU c auCACuU	1118	cgUgGCU A CAGGaGU

1118 CgUGGCU a CAggAgU
1141 CgCaGCU u gUGCUCG
1164 aCCUGgU U GCCAUCa
1202 UGuaaUU a UUUaUaC
1220 gGcAuCU c AgAAACu
1220 GGCAuCU C AGAAACu
1228 aGAaACU c UAgcaGG
1253 AaCaGGU a GUGgAAu
1331 AGgAGcU U GCUGCcc
1362 uUuUGaU C CCugGGA
1373 gGGaCUU c AUgguAA
1373 GgGACUU c AugguaA
1413 uUGUCAU u UGaccUC
1443 GUaaUGU a CcccGUG
1470 CACaUAU c CUaaaAu
1492 GugGUGU a uUGuAga
1497 GuAuUGU A gaAaUuA
1508 auUauUU a aUCcGCC
1508 AUuAuUU a auCCGcC
1523 cuGGGuU u CUaccUG

Table BXIII: Mouse CD40 Hammerhead Ribozyme Sequences

nt. Position	HH Ribozyme Sequence
18	CGAGGCA CUGAUGAGGCCGAAAGGCCGAA AGACACC
18	CGAGGCA CUGAUGAGGCCGAAAGGCCGAA AGACACC
24	CACAGCC CUGAUGAGGCCGAAAGGCCGAA AGGCAAA
38	AGCCCCA CUGAUGAGGCCGAAAGGCCGAA AGCGCGC
62	CUAGAUG CUGAUGAGGCCGAAAGGCCGAA ACCGCUG
62	CUAGAUG CUGAUGAGGCCGAAAGGCCGAA ACCGCUG
66	UGCCCUA CUGAUGAGGCCGAAAGGCCGAA AUGGACC
80	UGCACGU CUGAUGAGGCCGAAAGGCCGAA ACACACU
80	UGCACGU CUGAUGAGGCCGAAAGGCCGAA ACACACU
81	CUGCACG CUGAUGAGGCCGAAAGGCCGAA AACACAC
100	GUGGAGG CUGAUGAGGCCGAAAGGCCGAA ACUGUUU
126	UGGCACA CUGAUGAGGCCGAAAGGCCGAA AUCACAG
127	CUGGCAC CUGAUGAGGCCGAAAGGCCGAA AAUCACA
170	UCUUCUC CUGAUGAGGCCGAAAGGCCGAA AGAGCUG
208	GGCUGAG CUGAUGAGGCCGAAAGGCCGAA AUUCGCC
209	GGGCUGA CUGAUGAGGCCGAAAGGCCGAA AAUUCGC
233	GACAGCG CUGAUGAGGCCGAAAGGCCGAA AUCUCCC
267	AGCCCUU CUGAUGAGGCCGAAAGGCCGAA AUUGGGU
267	AGCCCUU CUGAUGAGGCCGAAAGGCCGAA AUUGGGU
275	UAACCCG CUGAUGAGGCCGAAAGGCCGAA AGCCCUU
275	UAACCCG CUGAUGAGGCCGAAAGGCCGAA AGCCCUU
276	UUAACCC CUGAUGAGGCCGAAAGGCCGAA AAGCCCU
281	CCUUCUU CUGAUGAGGCCGAAAGGCCGAA ACCCGAA
281	CCUUCUU CUGAUGAGGCCGAAAGGCCGAA ACCCGAA
314	AGGUACA CUGAUGAGGCCGAAAGGCCGAA ACAGUGU
354	GCCUCGC CUGAUGAGGCCGAAAGGCCGAA AUCUUG
386	AGCCAGG CUGAUGAGGCCGAAAGGCCGAA AUACAGG
394	AACUCCA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
394	AACUCCA CUGAUGAGGCCGAAAGGCCGAA AGCCAGG
395	UAACUCC CUGAUGAGGCCGAAAGGCCGAA AAGCCAG
429	CAGACGG CUGAUGAGGCCGAAAGGCCGAA AUCAGUG
434	GAUGACA CUGAUGAGGCCGAAAGGCCGAA ACGGUUU
434	GAUGACA CUGAUGAGGCCGAAAGGCCGAA ACGGUUU
441	GGCAGG CUGAUGAGGCCGAAAGGCCGAA AUGACAG
452	AGAAGCC CUGAUGAGGCCGAAAGGCCGAA ACUGGGC
452	AGAAGCC CUGAUGAGGCCGAAAGGCCGAA ACUGGGC
457	GGAGAAG CUGAUGAGGCCGAAAGGCCGAA AGCCGAC
458	UGGAGAA CUGAUGAGGCCGAAAGGCCGAA AAGCCGA
460	AUUGGAG CUGAUGAGGCCGAAAGGCCGAA AGAAGCC
461	GAUUGGA CUGAUGAGGCCGAAAGGCCGAA AAGAAGC
463	CUGAUUG CUGAUGAGGCCGAAAGGCCGAA AGAAGAA
472	AAGUGAU CUGAUGAGGCCGAAAGGCCGAA ACUGAUU
472	AAGUGAU CUGAUGAGGCCGAAAGGCCGAA ACUGAUU

479 UUUCGAA CUGAUGAGGCCGAAAGGCCGAA AGUGAUG
480 UUUUCGA CUGAUGAGGCCGAAAGGCCGAA AAGUGAU
481 CUUUUCG CUGAUGAGGCCGAAAGGCCGAA AAAGUGA
481 CUUUUCG CUGAUGAGGCCGAAAGGCCGAA AAAGUGA
492 CAGGGAU CUGAUGAGGCCGAAAGGCCGAA ACACUUU
560 CACAGAU CUGAUGAGGCCGAAAGGCCGAA ACAUUAG
563 AACCACA CUGAUGAGGCCGAAAGGCCGAA AUGACAU
572 GGGACUU CUGAUGAGGCCGAAAGGCCGAA AAACCAC
572 GGGACUU CUGAUGAGGCCGAAAGGCCGAA AAACCAC
577 CAUCCGG CUGAUGAGGCCGAAAGGCCGAA ACUUUAA
620 UGAUGAG CUGAUGAGGCCGAAAGGCCGAA AUGCCCA
626 AAAUGGU CUGAUGAGGCCGAAAGGCCGAA AUGAGGA
632 CCCCGAA CUGAUGAGGCCGAAAGGCCGAA AUGGUGA
632 CCCCGAA CUGAUGAGGCCGAAAGGCCGAA AUGGUGA
634 CACCCCG CUGAUGAGGCCGAAAGGCCGAA AAAUGGU
635 ACACCCC CUGAUGAGGCCGAAAGGCCGAA AAAAUGG
635 ACACCCC CUGAUGAGGCCGAAAGGCCGAA AAAAUGG
635 ACACCCC CUGAUGAGGCCGAAAGGCCGAA AAAAUGG
647 UGAUAUA CUGAUGAGGCCGAAAGGCCGAA AGAAACA
649 UUUGAUA CUGAUGAGGCCGAAAGGCCGAA AGAGAAA
651 UUUUUGA CUGAUGAGGCCGAAAGGCCGAA AUAGAGA
653 CCTUUUU CUGAUGAGGCCGAAAGGCCGAA AUUAAGA
735 CCGGGAU CUGAUGAGGCCGAAAGGCCGAA AUCUUCC
759 UGCACUG CUGAUGAGGCCGAAAGGCCGAA AGCAGCG
794 CCUGUGU CUGAUGAGGCCGAAAGGCCGAA ACAGGCU
794 CCUGUGU CUGAUGAGGCCGAAAGGCCGAA ACAGGCU
819 GAGAUGC CUGAUGAGGCCGAAAGGCCGAA ACUCUCU
824 GCACUGA CUGAUGAGGCCGAAAGGCCGAA AUGCGAC
826 CUGCACU CUGAUGAGGCCGAAAGGCCGAA AGAUGCG
876 GGGUUCA CUGAUGAGGCCGAAAGGCCGAA ACCAGGG
913 GGUCAGC CUGAUGAGGCCGAAAGGCCGAA AGCAGCC
997 AAAAAGC CUGAUGAGGCCGAAAGGCCGAA AGUUGAG
1003 AUCCUUA CUGAUGAGGCCGAAAGGCCGAA AAAGCAA
1003 AUCCUUA CUGAUGAGGCCGAAAGGCCGAA AAAGCAA
1023 GAUGCCC CUGAUGAGGCCGAAAGGCCGAA AGCUUUC
1048 UGGUAGA CUGAUGAGGCCGAAAGGCCGAA AUCACUG
1052 CACUUGG CUGAUGAGGCCGAAAGGCCGAA AGAUUUC
1081 GCAAGAC CUGAUGAGGCCGAAAGGCCGAA ACUCUGG
1084 GCAGCAA CUGAUGAGGCCGAAAGGCCGAA ACAACUC
1086 CCGCAGC CUGAUGAGGCCGAAAGGCCGAA AGACAAC
1097 UACAGUG CUGAUGAGGCCGAAAGGCCGAA ACGCCGC
1098 UUACAGU CUGAUGAGGCCGAAAGGCCGAA AACGCCG
1118 ACUCCUG CUGAUGAGGCCGAAAGGCCGAA AGCCACG
1118 ACUCCUG CUGAUGAGGCCGAAAGGCCGAA AGCCACG
1141 CGAGCAC CUGAUGAGGCCGAAAGGCCGAA AGCUGCG
1164 UGAUGGC CUGAUGAGGCCGAAAGGCCGAA ACCAGGU
1202 GUUAUAA CUGAUGAGGCCGAAAGGCCGAA AAUUAUA
1220 AGUUUCU CUGAUGAGGCCGAAAGGCCGAA AGAUGCC
1220 AGUUUCU CUGAUGAGGCCGAAAGGCCGAA AGAUGCC

1228	CCUGCUA	CUGAUGAGGCCGAAAGGCCGAA	AGUUUCU
1253	AUUCAC	CUGAUGAGGCCGAAAGGCCGAA	ACCUGUU
1331	GGGCAGC	CUGAUGAGGCCGAAAGGCCGAA	AGCUCCU
1362	UCCCAGG	CUGAUGAGGCCGAAAGGCCGAA	AUCAAAA
1373	UUACCAU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCC
1373	UUACCAU	CUGAUGAGGCCGAAAGGCCGAA	AAGUCCC
1413	GAGGUCA	CUGAUGAGGCCGAAAGGCCGAA	AUGACAA
1443	CACGGGG	CUGAUGAGGCCGAAAGGCCGAA	ACAUUAC
1470	AUUUUAG	CUGAUGAGGCCGAAAGGCCGAA	AUAUGUG
1492	UCUACAA	CUGAUGAGGCCGAAAGGCCGAA	ACACCAC
1497	UAAUUUC	CUGAUGAGGCCGAAAGGCCGAA	ACAAUAC
1508	GGCGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1508	GGCGGAU	CUGAUGAGGCCGAAAGGCCGAA	AAAUAAU
1523	CAGGUAG	CUGAUGAGGCCGAAAGGCCGAA	AACCCAG

Table BXIV: Human B7 Hairpin Ribozyme and Target Sequence

nt. Position	Hairpin Ribozyme Sequence		Substrate
286	ACAGGCAG	AGAA GAUGAC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	GUCAUCA GCC CUGCCUGU
291	GCRAAACA	AGAA GGGCUG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CAGCCCU GCC UGUUUUGC
295	AGGUGCAA	AGAA GGCAGG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CCUGCCU GUU UUGCACCUC
437	GCACCAAG	AGAA GAAAGA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UCUUUCA GCU CUUGGUGC
469	AACACCTUG	AGAA GAAGUG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CACUUCU GUU CAGGUGUU
518	GACACACAG	AGAA GCGUUG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CAACCCU GUC CUGUGGUC
540	AGTUCUUC	AGAA GAAACA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UGUUUCU GUU GAAGAGCU
596	ACAUCAUA	AGAA GCACCA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UGGUGCU GAC UAUAGAUGU
644	CAAAGAUG	AGAA GGUUCU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	AGAACCG GAC CAUCUUUG
702	GUGCCCTUC	AGAA GAUGGG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CCCAUCU GAC GAGGCGAC
795	GUAGGGAA	AGAA GCTUUG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CAAGCTU GAC UUCGCCUAC
819	AUTUCAAA	AGAA GAUAUA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UAUAUCU GAC UUUUGAAAU
939	UCUUGGGA	AGAA GUUGUG ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	CACAACA GUU UCCCAAGA
1012	ACACAUGA	AGAA GUGGUU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	AACCACA GCU UCAUGUGU
1055	AGUUGAAG	AGAA GAUUCA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UGAAUCA GAC CUUCAACU
1103	AGGAUGGG	AGAA GGUUAU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	AUAACCU GCU CCCAUCCU
1159	GUAGGUCA	AGAA GCAUAU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	AUAUGCU GCC UGACCUAC
1163	AGCAGUAG	AGAA GGCAGC ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	GCUGCCU GAC CUACUGCU
1171	UGGGGCAA	AGAA GUAGGU ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	ACCUACU GCU UUGCCCCA
1356	GUGGGURA	AGAA GCUUAA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UUUAGCU GUU UUAACCCAC
1395	UCAGCUUA	AGAA GAAAGA ACCAGAGAAACACACGUGUGGUAUUAUACCUUGUA	UCUUUCA GAU UAGGCTUGA

Table BXV: Mouse B7 Hairpin Ribozyme and Target Sequence

nt. Position	Hairpin Ribozyme Sequence	Substrate
74	AGAAUUGG AGAA GAGUGU ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	ACACUCU GUU CCAUUUCU
114	AUCCACCC AGAA GAUGCU ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	AGCAUCU GCC GGGUGGAU
154	AAUGCAGA AGAA GAGUUG ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	CAUCUCU GUU UCUCGAUU
265	CTUGCAUC AGAA GACAAU ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	AUUGUCA GUU GAUGCAGG
328	GACGAUUC AGAA GCACAA ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	UUGUGCU GCU GAUUCGUC
331	AAAGACGA AGAA GCAGCA ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	UGCUGCU GAU UCGUCUUU
356	UCAUCACAC AGAA GAAAGC ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	GUUCUCA GAU GUUGAUGA
373	CUGACUUG AGAA GUUGUU ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	AACAACU GUC CAAGUCAG
403	AACGGCAA AGAA GCAUAU ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	UAUUGCU GCC UUGCCGUU
481	CAUUGACA AGAA GCACCA ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	UGGUGCU GUC UGUCAUUG
529	CAUAUAAA AGAA GGUUCU ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	AGAACCG GAC UUUUAUUG
584	GUGCCCOG AGAA GAAAGG ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	CCUUUCA GAC CGGGGCAC
600	AACGACAC AGAA GUAUGU ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	ACAUACA GCU GUGUCGUU
677	GUAGAGAA AGAA GCUUUG ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	CAAAGCU GAC UUCUCUAC
741	GGAAACCA AGAA GGUAAU ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	AUUACCU GCU UUGCUUCC
1028	AUGACGAC AGAA GUUAUU ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	AAUAACA GUC GUCGUCAU
1077	UCUUCUGA AGAA GCUUCU ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	AGAAGCU GUU UCAGAAGA
1116	GAAGGUAA AGAA GUUGUU ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	AACAACA GCC UUAACCUUC
1153	GGAGACG AGAA GUUCAG ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	CUGAACA GAC CGUCUUCC
1157	UAAAGGAA AGAA GUCUGU ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	ACAGACC GUC UUCCUUUA
1178	CCCACAUG AGAA GAGAAG ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	CUUCUCU GUC CAUGUGGG
1246	UCCGAAAG AGAA GCUAGC ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	GCUAGCU GAU CUUUCGGA
1523	CAGAAAAG AGAA GGCCUC ACCAGAGAAACACACGUGUGGUACAUUACCUUGGUA	GAGGCCU GCC CUUUUCUG

Table BXVI: Human B7-2 Hairpin Ribozyme and Target Sequences

nt. Position	HP Ribozyme Sequences		Substrate
25	GUUACAGC	AGAA GAGAAG	CUUCUCU GCU GCUGUAAC
28	CCUGUAC	AGAA GCAGAG	CUCUGCU GCU GUAACAGG
57	CCCACUC	AGAA GUGUGU	ACACACG GAU GAGUGGGG
162	CACCAGAG	AGAA GGAAGG	CCUUCU GCU CUCUGGUG
175	UUCAGAGG	AGAA GCACCA	UGGUGCU GCU CCUCUGAA
214	CAUGGCAG	AGAA GCAGUC	GACUGCA GAC CUGCCAU
380	CAGGCUCC	AGAA GUCCGA	UCCGACA GUU GGACCCUG
408	UGUCCUUG	AGAA GAAGAU	AUCUACA GAU CAAGGACA
480	CAGAAUUC	AGAA GGUGGA	UCCACCA GAU GAUUCUG
575	UAUAGAUG	AGAA GGUCAA	UUGACCU GCU CAUCUAUA
710	AACAGACA	AGAA GAUGGA	UCCAUCA GCU UGUCUGU
718	GGGAUGA	AGAA GACAAG	CUUGUCU GUU UCAUUC
730	CUCGUAC	AGAA GGGAU	AUUCUCCU GAU GUUACGAG
783	AAGAUAAA	AGAA GCGUCU	AGACGCG GCU UUUUAUCU
825	CUGGGGA	AGAA GAGGGU	ACCCUCA GCC UCCCCCAG
835	GGAAUGUG	AGAA GGGGGA	UCCCCCA GAC CACAUUCC
856	GGAAUAC	AGAA GUAAUC	GAUUA CA GCU GUACUUC
896	UAGAAUUA	AGAA GAAAAC	GUUUUCU GUC UAAUUCUA
930	AGUUGGA	AGAA GCUUCU	AGAAGCG GCC UCGCAACU
987	UUUUCUUG	AGAA GUUCAC	GUGAACA GAC CAAGAA
1027	UGGGCUUC	AGAA GAUCUU	AAGAUCU GAU GAAGCCCA

Table BXVII: Mouse B7-2 Hairpin Ribozyme and Target Sequences

nt. Position	HP Ribozyme Sequences	Substrate
10	UCUUAACC AGAA GCUUGC ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	GCAAGCA GAC GCUUAAGA
42	UUGUUCAA AGAA GUGCUG ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	CAGCAGC GAC UUGAACAA
56	CUACAGGA AGAA GGUUGU ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	ACAACCA GAC UCCUGUAG
108	CAUGGUGC AGAA GGGGUC ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	GACCCCA GAU GCACCAUG
146	AUCAGCAA AGAA GUCACA ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	UGUGACA GUC UUGCUGAU
154	CAUCUGAG AGAA GCAAGA ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	UCUUGCU GAU CUCAGAUG
161	GAAACAGC AGAA GAGAUC ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	GAUTUCA GAU GCUGUUUC
167	UCCACCGA AGAA GCAUCU ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	AGAUGCU GUU UCCGUGGA
211	AUGGGCAC AGAA GAUAUG ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	CAUAUCU GCC GUGCCCAU
400	UGUCCUUG AGAA GAACAU ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	AUGUUCA GAU CNAAGACA
679	AGAUACUG AGAA GUUCUG ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	CAGAACU GUU CAGUAUCU
696	AAGAGAGA AGAA GUUGGA ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	UCCNACA GCC UCUCUCUU
716	CACACACC AGAA GGGAAU ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	AUUCOCG GAU GGUGUGUG
737	ACACACAC AGAA GUCAUA ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	UAUGACC GUU GUGUGUGU
839	GUAACTUG AGAA GUAAUC ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	GAUUAAC GCU UCAGUUAU
874	CAUUGAUG AGAA GCAUCA ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	UGAUGCU GCU CAUCAUUG
907	GCCUGCUA AGAA GAUUCG ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	CGAUACA GCC UAGCAGGC
929	AACUUAAG AGAA GUGUUG ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	CAACACA GCC UCUAAGUU
1115	UUCCAAUC AGAA GAGAAC ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	GUUCUCA GCU GAUUGGAA
1118	GAUUAUCC AGAA GCUAG ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	CUCAGCU GAU UGGAUAUC
1133	AAUUAUUC AGAA GUAGAA ACCAGAGAAACACACGUGUGGUAACAUUACCUUGUA	UUCUACA GUU GAAUAAUU

Table BXVIII : Human CD40 Hairpin Ribozyme and Target Sequences

nt. Position	Hairpin Ribozyme Sequences		Substrate
26	GACCAGGC	AGAA GGACCA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	UGGUCCU GCC GCUUGGUC
29	UGAGACCA	AGAA GCAGGA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	UCCUGGC GCC UGGUCUCA
58	ACUGCAGA	AGAA GACGAA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	UUCGUCU GCC UCUGCAGU
84	GGUCAGCA	AGAA GOCCTCA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	UGGGGCU GCU UGUGACCC
91	GGACAGCG	AGAA GCNAGC ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	GCUUGCU GAC CGCUGUCC
95	GGUUGGAC	AGAA GUCAGC ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CTUGACC GCU GUCCAUCC
98	UCUGGAUG	AGAA GCGGUC ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	GACCGCU GUC CAUCCAGA
159	GCACAAAG	AGAA GCACUG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CAGUGCU GUU CUUUGUGC
414	CGAGCAUG	AGAA GUGCAG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CUGACCC GCU CAUGCUCG
429	GACCCCAA	AGAA GGGCGA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	UOGCCCG GCU UUGGGGUC
445	CUGUAGCA	AGAA GCUUGA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	UCAAGCA GAU UGCUACAG
483	GCCGACUG	AGAA GGGCUC ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	GAGCCCU GCC CAGUOGGC
488	AAGAAGCC	AGAA GGGCAG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CUGCCCA GUC GGCUCUCU
492	GGAGAAGA	AGAA GACTUG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CCAGUGG GCU UCUUCUCC
515	UUUUCGAA	AGAA GAUGAC ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	GUCAUCU GCU UUCGAAAA
593	CAGACAAC	AGAA GUCTUG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CAAGACU GAU GUUGUCUG
619	GGGCTUC	AGAA GAUCCU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	AGGAUCG GCU GAGAGGCC
661	GGUUGGCA	AGAA GGAUCC ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	GGAUCCU GUU UGCCAUCC
764	GGAGAUC	AGAA GGAAAA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	UUUCCOC GAC GAUUCUCC
788	ACUGGAGC	AGAA GUGUUG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CAACACU GCU GCUCCAGU
791	UGCACUGG	AGAA GCAGUG ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	CACUGCU GCU CCAGUGCA
924	CUCUGGCC	AGAA GCCUGU ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	ACAGGCA GUU GGCCAGAG
946	CCUGCAGC	AGAA GCACCA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	UGGUGCU GCU GCUGCAGG
949	ACCCUCUG	AGAA GCAGCA ACCAGAGAAACACACGUGUGUGGUACAUUACCUUGUA	UGCUGCU GCU GCAGGGGU

Table BXIX: Mouse CD40 Hairpin Ribozyme and Substrate Sequences

nt. Position	HP Ribozyme Sequences	Substrate
25	GGCGCAC AGAA GAGGCA ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	UGCCUUG GCU GUGGCGC
45	UGUCAACA AGAA GCGCCA ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	UGGGGCU GCU UGUUGACA
59	CCUAGAUG AGAA GCUGUC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GACAGG GUC CAUCUAGG
144	GCUGUCA AGAA GCUUCC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GGAAGCC GAC UGACAAGC
164	UUCUCAG AGAA GUGCAG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CUGCACA GCU CUUGAGAA
212	UUCCACUG AGAA GAGAAU ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	AUUCUCA GCC CAGUGGAA
311	CAGGUACA AGAA GUGUCU ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	AGACACTU GUC UGUACCUG
431	GGUUGACA AGAA GUUAUA ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	UGAUACC GUC UGUCAUCC
444	GCCGACUG AGAA GGGUUG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CAUCCCU GCC CAGUGGCG
449	AAGAAGCC AGAA GGGCAG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CUGCCCA GUC GGUUCUCU
453	GGAGAAGA AGAA GACTUG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CCAGUG GCU UCUUCUCC
550	UGACAUUA AGAA GACTUG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CGAGUCA GAC UAAUGUCA
580	GGGCUCCG AGAA GGGACU ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	AGUCCCG GAU GCGAGCCC
592	GAUUGACC AGAA GGGCUC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GAGCCCU GCU GGUCAUUC
605	CCCAUCAC AGAA GGAAUG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CAUUCU GUC GUGAUGGG
701	UGCCGUCG AGAA GCAGGG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CCCUGCG GCU CGACGGCA
752	ACUGGAGC AGAA GUGUUA ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	UAAACC GCU GCUCCAGU
755	UGCACUUG AGAA GCGGUG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CACCGCU GCU CCAGUGCA
787	GUGUGACA AGAA GACACC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GGUGUCA GCC UGUACACAC
890	CTUCCAAA AGAA GUUCCA ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	UGGAACTU GCU UUUGGAGG
909	GGUCAGCA AGAA GCCAUC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GAUGGCU GCU UGCUGACC
916	UUCAAAAG AGAA GCAAGC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GCUUGCU GAC CUUUUGAA
975	UGACAGGG AGAA GGC AUG ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	CAUGGCU GCC CCCUGUCA
1137	CGAGCACA AGAA GCGGGC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GCCCCGA GCU UGUUCUUG
1276	GUUUUAAA AGAA GUUUCU ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	AGAAACA GCU UUUUAAAC
1334	CGGGUUUG AGAA GCAAGC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GCUUGCU GCC CAAACCCG
1352	GGAUCAAA AGAA GGUUAC ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	GUUACCU GAU UUUGAUCC
1512	AAACCCAG AGAA GAUUA ACCAGAGAAACACACGUGUGGUACAUUACCUUGUA	UUAAUCC GCC CUGGGUUU

Table CII: 2.5 μ mol RNA Synthesis Cycle

Reagent	Equivalents	Amount	Wait Time*
Phosphoramidites	6.5	163 μ L	2.5
S-Ethyl Tetrazole	23.8	238 μ L	2.5
Acetic Anhydride	100	233 μ L	5 sec
N-Methyl Imidazole	186	233 μ L	5 sec
TCA	83.2	1.73 mL	21 sec
Iodine	8.0	1.18 mL	45 sec
Acetonitrile	NA	6.67 mL	NA

* Wait time does not include contact time during delivery.

Table EVII: Deprotection of a 36 mer all ribo oligo using 70% ethylamine in aqueous. The data are as follows upon HPLC reprocessing:

Sample	OD's	% Full Length Product (FLP)	% frontside	%backside
MA 10'@65°	0.984	14.5073	71.6740	13.8186
MA 10'@65°	1.125	18.9269	67.8006	13.2725
EA rt 10'	0.925	16.5804	66.8186	16.6010
EA rt 10'	0.920	15.7421	67.5794	16.6785
EA rt 30'	0.971	17.4694	67.6782	14.8525
EA rt 30'	0.794	15.7587	69.8084	14.4329
EA 40° 10'	0.819	18.0827	66.4937	15.4236
EA 40° 10'	0.986	17.5763	66.7865	15.6372
EA 40° 15'	0.877	18.7963	67.0064	14.1999
EA 40° 15'	0.911	18.7808	70.7306	10.4885
EA 55° 10'	1.001	17.8810	66.4703	15.6487
EA 55° 10'	1.023	19.1069	68.6706	12.2225

Claims

1. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein
5 said nucleic acid comprises a 2'-C-allyl modification at position No. 4 of said nucleic acid, and wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'- end modification.
2. The enzymatic nucleic acid of claim 1, wherein said nucleic acid
10 comprises a 3'-3' linked inverted ribose moiety at said 3' end.
3. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises a 2'-amino modification at position No. 4 and/or at position No. 7 of said nucleic acid, wherein said nucleic acid
15 comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.
4. An enzymatic nucleic acid having a hammerhead motif, wherein said nucleic acid comprises of at least five ribose residues, and wherein
20 said nucleic acid comprises a non-nucleotide substitution at position No. 4 and/or at position No. 7 of said nucleic acid molecule, wherein said nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.
- 25 5. An enzymatic nucleic acid which cleaves target mRNA having a sequence selected from SEQ. ID. NOS. 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, and 281, wherein said nucleic acid comprises of at least five ribose residues, and wherein said nucleic acid comprises a 6-methyl
30 uridine substitution at position No. 4 and/or at position No. 7 of said nucleic acid molecule, wherein said nucleic acid comprises at least

ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 3'-3' linked inverted ribose or thymidine moiety at its 3' end.

- 5 6. The enzymatic nucleic acid which cleaves target mRNA having a sequence selected from SEQ. ID. NOS. 34, 35, 57, 125, 126, 127, 128, 129, 140, 162, 170, 179, 188, 223, 224, 236, 245, 246, 256, 259, 260, and 281, wherein said nucleic acid comprises of at least five ribose residues, wherein said nucleic acid comprises a 2'-C-allyl modification at position No. 4 of the said nucleic acid, wherein said
- 10 nucleic acid comprises at least ten 2'-O-methyl modifications, and wherein said nucleic acid comprises a 2'-3' linked inverted ribose or thymidine moiety at its 3' end.
- 15 7. The enzymatic nucleic acid of any one of claims 1-6, wherein said nucleic acid comprises phosphorothioate linkages at least three of the seven 5' terminal nucleotides.
8. Nucleic acid molecule which blocks synthesis and/or expression of an mRNA encoding B7-1, B7-2, B7-3 and/or CD40.
9. The nucleic acid of claim 8, wherein said molecule is an enzymatic nucleic acid molecule.
- 20 10. The nucleic acid molecule of claim 9, wherein, the binding arms of said enzymatic nucleic acid contain sequences complementary to the nucleotide base sequences in any one of Tables BII, BIV, BVI, BVIII, BX, BXII, BXIV, BXV, BXVI, BXVII, BXVIII and BXIX.
- 25 11. The nucleic acid molecule of claims 9 or 10, wherein said nucleic acid molecule is in a hammerhead motif.
12. The enzymatic nucleic acid molecule of claim 9 or 10, wherein said nucleic acid molecule is in a hairpin, hepatitis Delta virus, group I intron, VS nucleic acid or RNaseP nucleic acid motif.

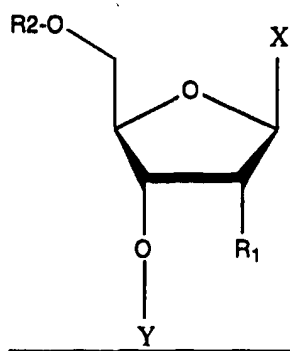
13. The enzymatic nucleic acid molecule of any of claims 9 or 10, wherein said ribozyme comprises between 12 and 100 bases complementary to the RNA of said region.
- 5 14. The enzymatic nucleic acid of claim 13, wherein said ribozyme comprises between 14 and 24 bases complementary to the RNA of said region.
- 15 15. Enzymatic nucleic acid molecule consisting essentially of any ribozyme sequence selected from those shown in Tables BIII, BV, BVI, BVII, BIX, BXI, BXIII, BXIV, BXV, BXVI, BXVII, BXVIII.
- 10 16. A mammalian cell including an enzymatic nucleic acid molecule of any of claims 8 or 9.
17. The cell of claim 16, wherein said cell is a human cell.
- 15 18. An expression vector comprising nucleic acid encoding the enzymatic nucleic acid molecule of any of claims 9 or 10, in a manner which allows expression and/or delivery of that enzymatic RNA molecule within a mammalian cell.
19. A mammalian cell including an expression vector of claim 18.
- 20 20. The cell of claim 19, wherein said cell is a human cell.
- 20 21. A method for treatment of a patient having a condition associated with the level of B7-1, B7-2, B7-3 and/or CD40, wherein the patient, tissue donor or population of corresponding cells is administered a therapeutically effective amount of an enzymatic nucleic acid molecule of claims 8, 9 or 10.
- 25 22. A method for treatment of a condition related to the level of B7-1, B7-2, B7-3 and/or CD40 activity by administering to a patient an expression vector of claim 21.
23. The method of claims 21 or 22, wherein said patient is a human.

24. A method for inducing tolerance in a recipient to alloantigen of a donor comprising treating antigen presenting cells from a donor with nucleic acid of claim 8 or 9, and infusion of said treated antigen presenting cells into said recipient.
- 5 25. A method for enhancing graft tolerance comprising contacting a nucleic acid of claims 8 or 9 with cells of said graft prior to transplantation.
26. A method for treatment of an autoimmune disease, comprising contacting an antigen presenting cell of a patient with a nucleic acid of claims 8 or 9.
- 10 27. The method of claim 26, wherein said cells are contacted *ex vivo* with said nucleic acid.
28. The method of claim 26, wherein said cells are contacted with autoantigen characteristic of said disease.
- 15 29. The method of claim 28, wherein said cells are reinfused into said patient.
- 20 30. Enzymatic nucleic acid having at least one modified base substitution, wherein said base substitution is selected from a group comprising pyridin-4-one, pyridin-2-one, phenyl, pseudouracil, 2, 4, 6-trimethoxy benzene, 3-methyluracil, dihydrouracil, naphthyl, 6-methyl-uracil and aminophenyl.
31. The enzymatic nucleic acid of any of claim 30, wherein said nucleic acid has a hammerhead motif.
32. Mammalian cell comprising an enzymatic nucleic acid molecule of and of claims 30-31.
- 25 33. The enzymatic nucleic acid of claim 31, wherein said nucleic acid includes said modified base substitutions at position 4 or at position 7.
34. The ribozyme of claim 33, wherein said substitution is 6-methyl uracil.
35. The ribozyme of claim 33, wherein said substitution is pyridin-4-one.

36. The ribozyme of claim 33, wherein said substitution is phenyl.
37. The ribozyme of claim 33, wherein said substitution is pyridin-2-one.
38. The ribozyme of claim 33, wherein said substitution is pseudouracil.
- 5 39. The ribozyme of claim 33, wherein said substitution is 2, 4, 6-trimethoxy benzene.
40. The ribozyme of claim 33, wherein said substitution is dihydrouracil.
41. The ribozyme of claim 33, wherein said substitution is 3-methyluracil.
42. The ribozyme of claim 33, wherein said substitution is naphthyl.
43. The ribozyme of claim 33, wherein said substitution is aminophenyl.
- 10 44. 2'-deoxy-2'-alkylnucleoside.
45. 2'-deoxy-2'-alkylnucleotide.
46. Oligonucleotide comprising one or more 2'-deoxy-2'-alkylnucleotides.
47. Enzymatic nucleic acid comprising a 2'-deoxy-2'-alkylnucleotide.
- 15 48. Method for producing an enzymatic nucleic acid molecule having enhanced activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one nucleotide having at its 2'-position an alkyl group.
49. 2'-deoxy-2'-alkylnucleotide triphosphate.
- 20 50. Method for synthesis of a 2'-C-allyl derivative from a 5'-O-DMT-3'-O-TBDMS-base comprising the steps of:
- (a) phenoxytriacarbonylation of 5'-O-DMT-3'-O-TBDMS-base to yield a thioester, replacing a 2' hydroxyl group with a phenoxythiocarbonyl group, and

(b) Heck acylation of said thioester to form a 2'-C-allyl derivative in which said 2'-phenoxythiocarbonyl group is replaced with said 2'-C-alkyl group to yield said 2'-C-allyl derivative.

51. A compound having the formula:



5

wherein, R1 represents 2'-O-alkylthioalkyl or 2'-C-alkylthioalkyl; X represents a base or H; Y represents a phosphorus-containing group; and R2 represents O, DMT or a phosphorus-containing group.

52. Oligonucleotide comprising one or more compounds of claim 51.

10 53. Enzymatic nucleic acid comprising a compound of claim 51.

54. The compound of claim 51, wherein said compound is in the form of a triphosphate.

55. Enzymatic nucleic acid of claim 53 wherein said nucleic acid is in a hammerhead motif.

15 56. Enzymatic nucleic acid of claim 53, wherein said nucleic acid is in a hairpin, hepatitis delta virus, group I intron, VS RNA or RNase P RNA motif.

57 Enzymatic nucleic acid of claim 55, wherein said hammerhead ribozyme has positions 4 and/or 7 substituted with 2'-O-methylthiomethyl.

20

58. Enzymatic nucleic acid of claim 55 or 57, wherein one monomer in stem II of said hammerhead is substituted with at least one 2'-O-methylthiomethyl.
59. Enzymatic nucleic acid of claim 55 or 56, wherein said nucleic acid is substituted at one or more positions with 2'-O-methylthiophenyl.
60. A mammalian cell comprising a compound of any one of the claims 51-59.
61. The cell of claim 60, wherein said cell is a human cell.
62. Method for producing an enzymatic nucleic acid molecule having activity to cleave an RNA or single-stranded DNA molecule, comprising the step of forming said enzymatic molecule with at least one position having at its 2'-position an 2'-O-alkylthioalkyl and/or 2'-C-alkylthioalkyl group.
64. Hammerhead ribozyme having a non-nucleotide in the catalytic core in a site selected from the group consisting of the normally occurring uracil at position 4 and 7.
65. Hammerhead ribozyme having a stem II and a loop II, wherein said loop II comprises a non-nucleotide.
66. Hammerhead ribozyme having a non-nucleotide at its 3' end.
67. A mammalian cell comprising an enzymatic nucleic acid molecule of any one of the claims 64-67.
68. The cell of claim 67, wherein said cell is a human cell.
69. Method of synthesis of abasic ribonucleoside mimetics described in figure 58.
70. A method for the deprotection of RNA comprising the step of providing aqueous ethylamine (EA) at between 25°C - 60°C for 5 to 30 minutes to remove any exocyclic amino protecting groups from protected RNA.

71. The method of claim 70 wherein, said ethylamine is provided at 40°C for 10 minutes.
72. The method of claim 70 wherein, said ethylamine is provided at 55°C for 10 minutes.
- 5 73. The method of claim 70, further comprising deprotection of RNA alkylsilyl protecting groups comprising, contacting said groups with anhydrous triethylamine•hydrogen fluoride (aHF•TEA) trimethylamine or diisopropylethylamine at between 60 °C-70 °C for 0.25-24 h.
- 10 74. The method of any one of claims 70-73 wherein, said RNA is an enzymatic RNA.
75. Method for synthesis of an enzymatic nucleic acid, comprising the steps of:
- 15 providing a 3' and a 5' portion of said enzymatic nucleic acid having independent chemically reactive groups at the 5' and 3' positions, respectively, under conditions in which a covalent bond is formed between said 3' and 5' portions by said chemically reactive groups, said bond being selected from the group consisting of, disulfide, morpholino, amide, ether, thioether, amine, a double bond, sulfonamide, ester, carbonate, hydrazone, said bond not being a
- 20 natural bond formed between a 5' phosphate group and a 3' hydroxyl group.
76. The method of claim 75, wherein said nucleic acid has a hammerhead motif and said 3' and 5' positions each have said chemically reactive groups in or immediately adjacent to the stem II region.
- 25 77. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{SH}$ and the other chemically reactive group is $(\text{CH}_2)_n\text{SH}$, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 30 78. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{NH}_2$ and the other chemically reactive group is ribose, wherein

each n independently is an integer from 0 to 10 inclusive and may be the same or different.

- 5 79. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{NH}_2$ and the other chemically reactive group is COOH , wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 10 80. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{X}$ and the other chemically reactive group is $(\text{CH}_2)_n\text{OH}$ or $(\text{CH}_2)_n\text{SH}$; wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different; X is halogen.
- 15 81. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{NH}_2$ and the other chemically reactive group is CHO , wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 20 82. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{PPh}_3$ and the other chemically reactive group is CHO , wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 25 83. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{NH}_2$ and the other chemically reactive group is $(\text{CH}_2)_n\text{SO}_2\text{Cl}$, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 30 84. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{OH}$ and the other chemically reactive group is COOH , wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
85. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{COH}$ and the other chemically reactive group is $(\text{CH}_2)_n\text{NH}_2$, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.

86. The method of claim 75, wherein one said chemically reactive group is $(\text{CH}_2)_n\text{COX}$ and the other chemically reactive group is $(\text{CH}_2)_n\text{OH}$, wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different.
- 5 87. The method of claim 78, wherein said conditions include provision of NaIO_4 in contact with said ribose, and subsequent provision of NaBH_4 or NaCNBH_3 .
88. The method of claim 79, wherein said conditions include provision of a coupling reagent.
- 10 89. A mixture comprising 5' and 3' portions of an enzymatic nucleic acid having a 3' and 5' chemically reactive group respectively selected from the group consisting of $(\text{CH}_2)_n\text{SH}$, $(\text{CH}_2)_n\text{NH}_2$, ribose, COOH , $(\text{CH}_2)_n\text{X}$, $(\text{CH}_2)_n\text{PPh}_3$, CHO , $(\text{CH}_2)_n\text{SO}_2\text{Cl}$, $(\text{CH}_2)_n\text{COX}$, $(\text{CH}_2)_n\text{X}$, $(\text{CH}_2)_n\text{OH}$, $(\text{CH}_2)_n\text{COH}$, and $(\text{CH}_2)_n\text{SH}$; wherein each n independently is an integer from 0 to 10 inclusive and may be the same or different and X is halogen.
- 15
90. The method of claim 75, wherein one said chemically reactive group is linking group-SH and the other chemically reactive group is linking group-SH, wherein each linking group may be the same or different.
- 20 91. The method of claim 75, wherein one said chemically reactive group is linking group- NH_2 and the other chemically reactive group is ribose.
92. The method of claim 75, wherein one said chemically reactive group is linking group- NH_2 and the other chemically reactive group is COOH .
- 25 93. The method of claim 75, wherein one said chemically reactive group is linking group-X and the other chemically reactive group is linking group-OH or linking group-SH; wherein each linking group may be the same or different; X is halogen.
94. The method of claim 75, wherein one said chemically reactive group is linking group- NH_2 and the other chemically reactive group is CHO .

95. The method of claim 75, wherein one said chemically reactive group is linking group-PPh₃ and the other chemically reactive group is CHO.
96. The method of claim 75, wherein one said chemically reactive group is linking group-NH₂ and the other chemically reactive group is linking group-SO₂Cl, wherein each linking group may be the same or different.
97. The method of claim 75, wherein one said chemically reactive group is linking group-OH and the other chemically reactive group is COOH.
98. The method of claim 75, wherein one said chemically reactive group is linking group-COH and the other chemically reactive group is linking group-NH₂, wherein each linking group may be the same or different.
99. The method of claim 75, wherein one said chemically reactive group is linking group-COX and the other chemically reactive group is linking group-OH, wherein each linking group may be the same or different.
100. The method of claim 91, wherein said conditions include provision of NaIO₄ in contact with said ribose, and subsequent provision of NaBH₄ or NaCNBH₃.
101. The method of claim 100, wherein said conditions include provision of a coupling reagent.
102. A mixture comprising 5' and 3' portions of an enzymatic nucleic acid having a 3' and 5' chemically reactive group respectively selected from the group consisting of linking group-SH, linking group-NH₂, ribose, COOH, linking group-X, linking group-PPh₃, CHO, linking group-SO₂Cl, linking group-COX, linking group-X, linking group-OH, linking group-COH, and linking group-SH; wherein each linking group may be the same or different and X is halogen.
103. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said

stem comprises at least 8 base pairs wherein said molecule is transcribed by a RNA polymerase II promoter system.

5 104. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is transcribed by a U6 small nuclear RNA promoter system.

10 105. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is transcribed by an adenovirus VA1 RNA promoter system.

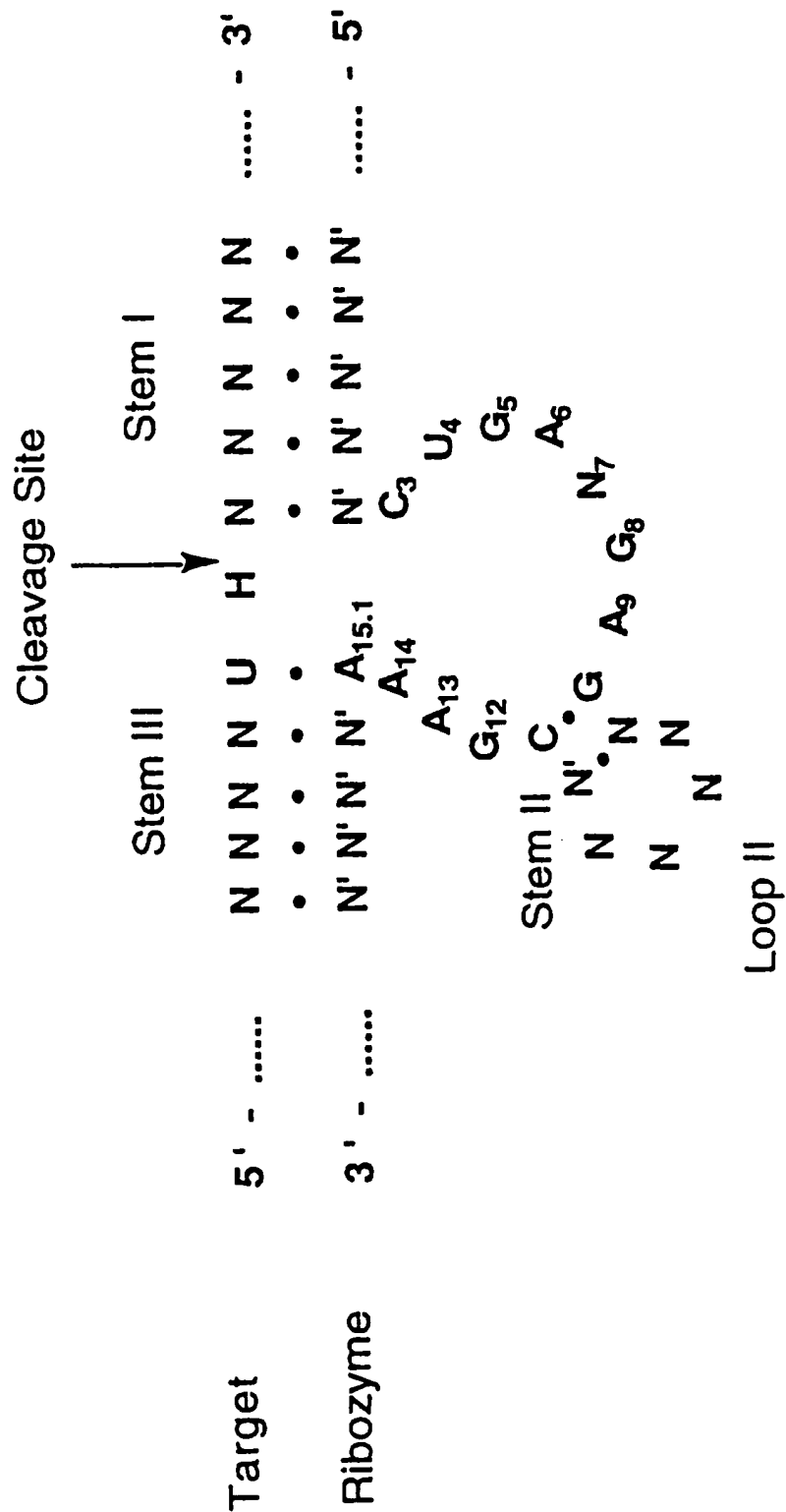
15 106. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said molecule is a chimeric adenovirus VA1 RNA.

20 107. A transcribed non-naturally occurring RNA molecule, comprising a desired therapeutic RNA portion, wherein said molecule comprises an intramolecular stem formed by base-pairing interactions between a 3' region and 5' complementary nucleotides in said RNA, wherein said stem comprises at least 8 base pairs, wherein said intramolecular stem is separated from said desired RNA by a spacer sequence.

25 108. The RNA molecule of claim 107, wherein said spacer sequence is about 5-50 nucleotides.

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FIG. 1.



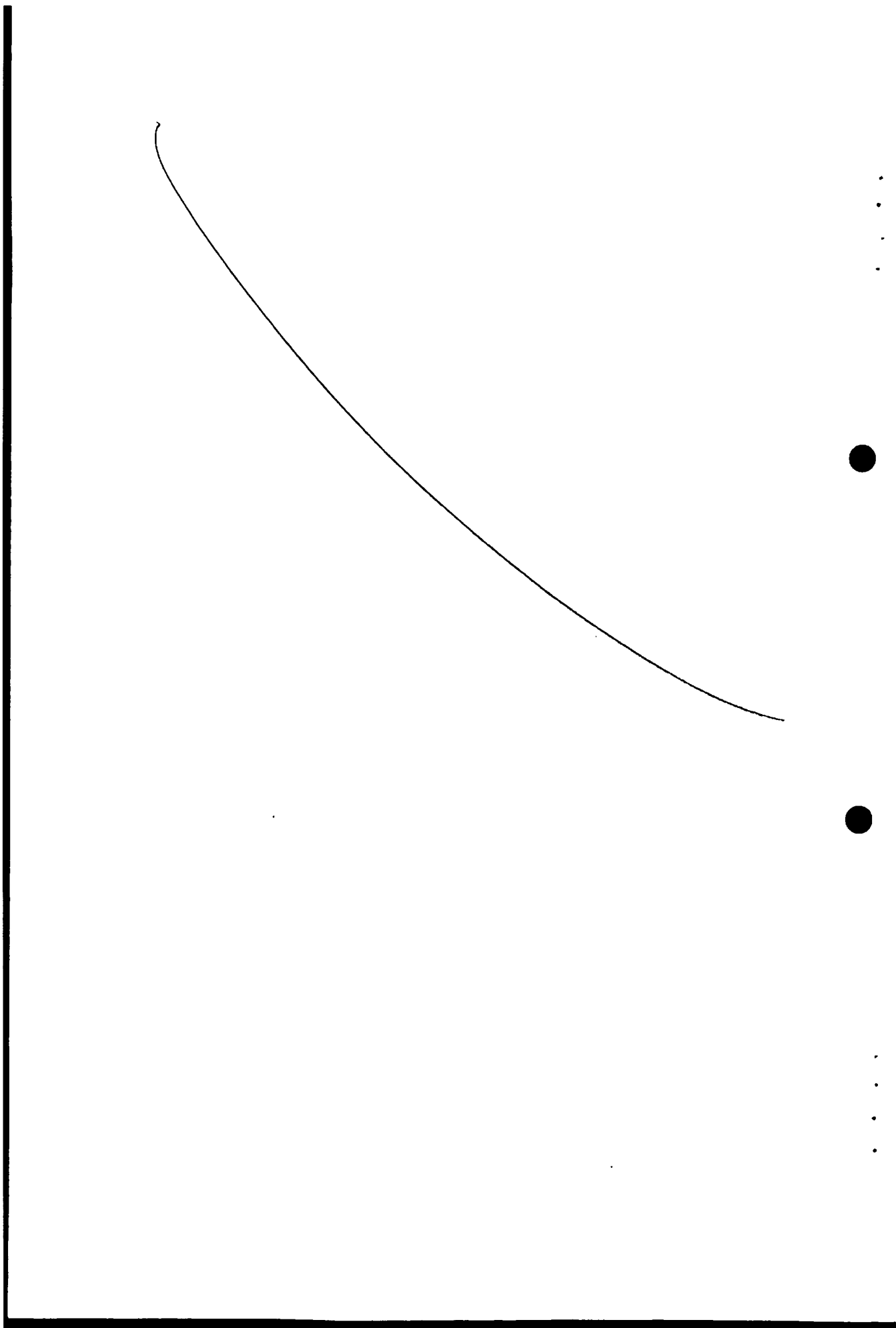


FIG. 2b.

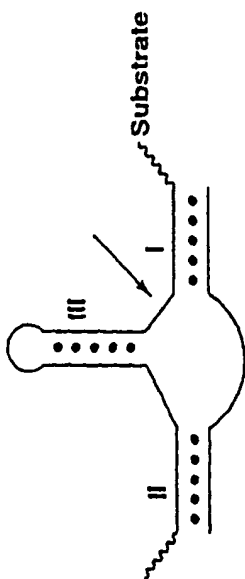


FIG. 2a.

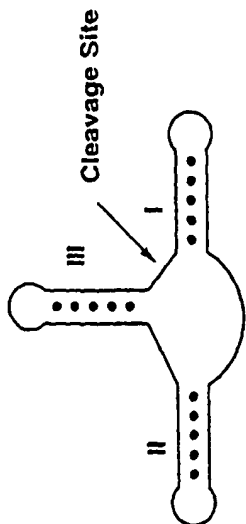


FIG. 2d.

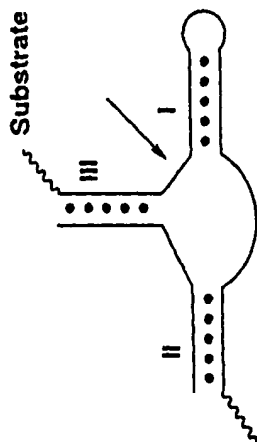
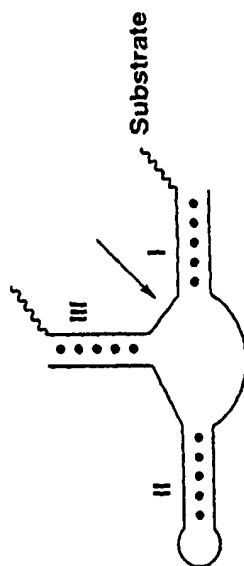
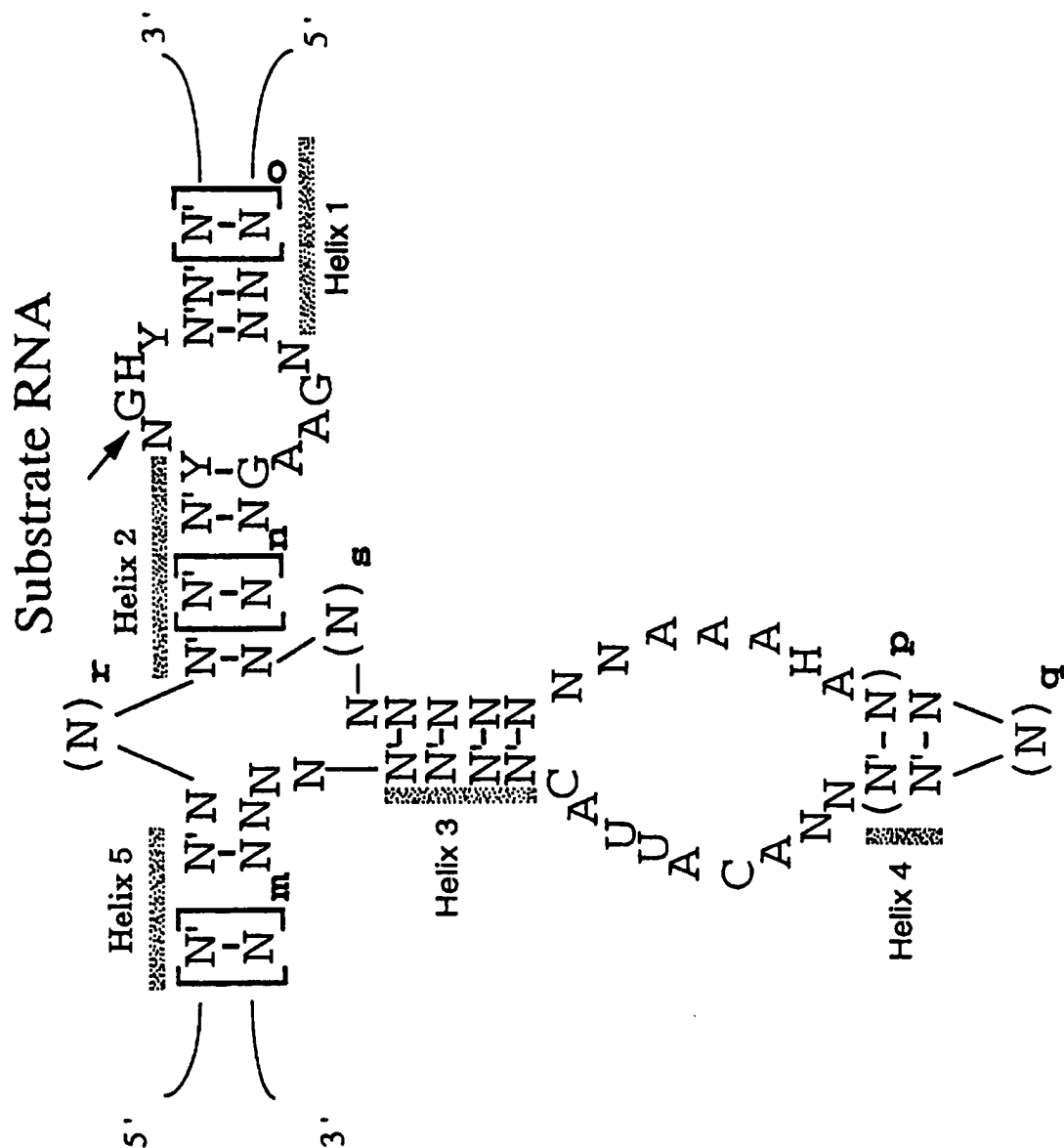


FIG. 2c.



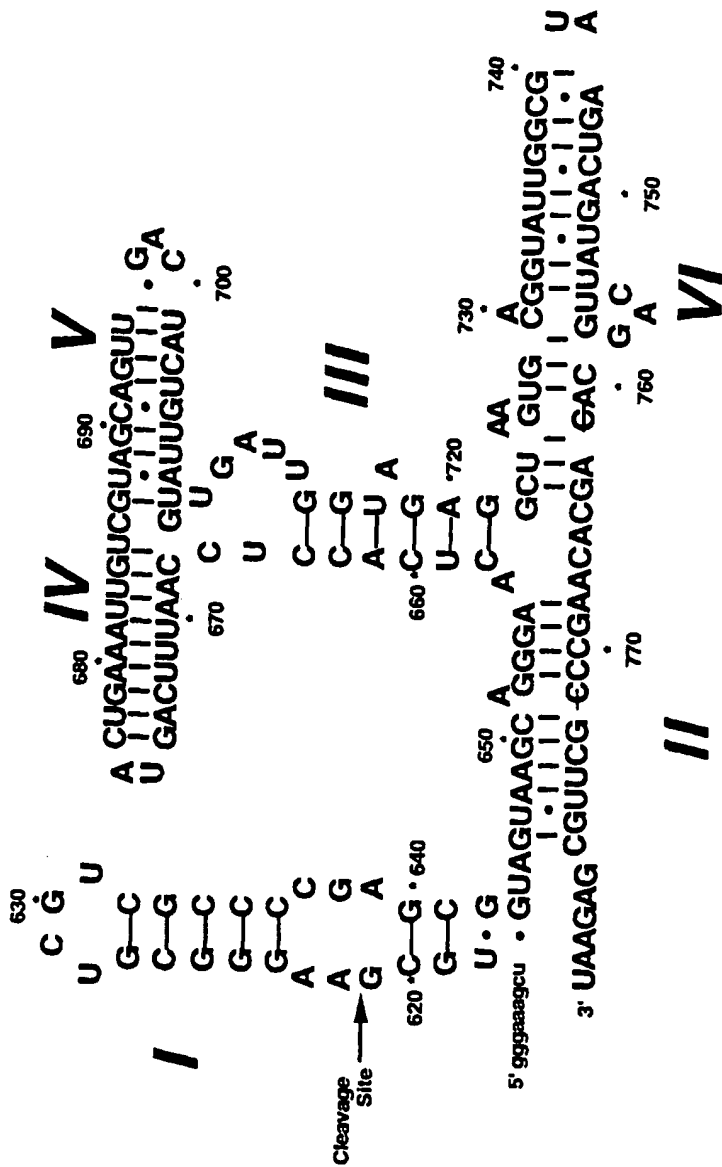
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FIG. 3.



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FIG. 5.



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FIG. 6b.

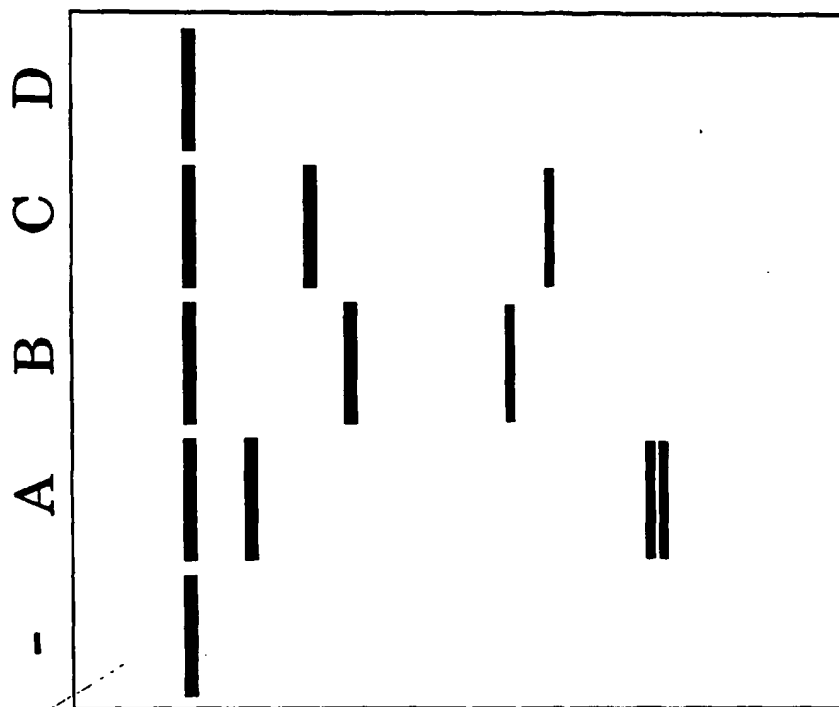
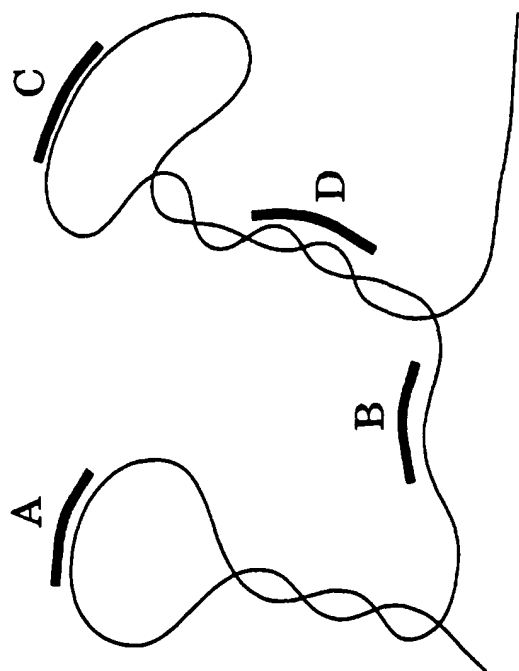


FIG. 6a.



- Body-labeled transcript (not purified)
- DNA oligo (10 nM, 100 nM and 1000 nM)
- RNase H (0.08 - 1.0 u/μl)
- 37°C, 10 min

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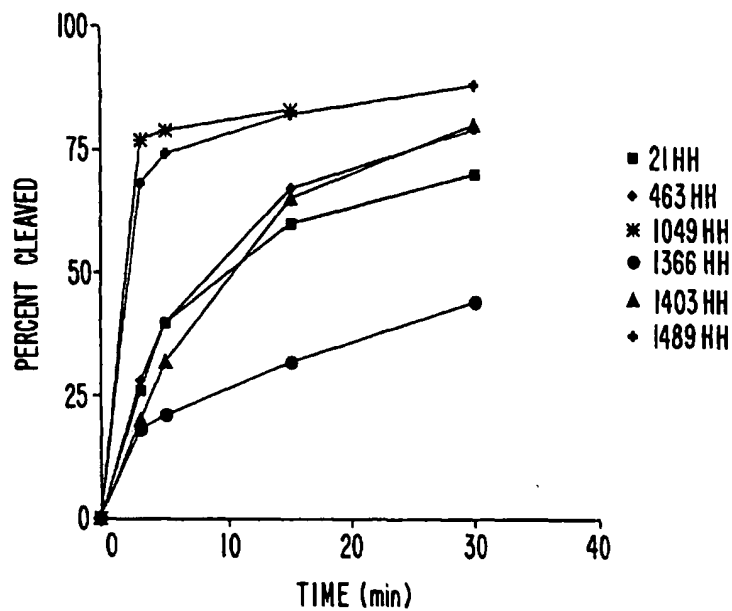


FIG. 7.

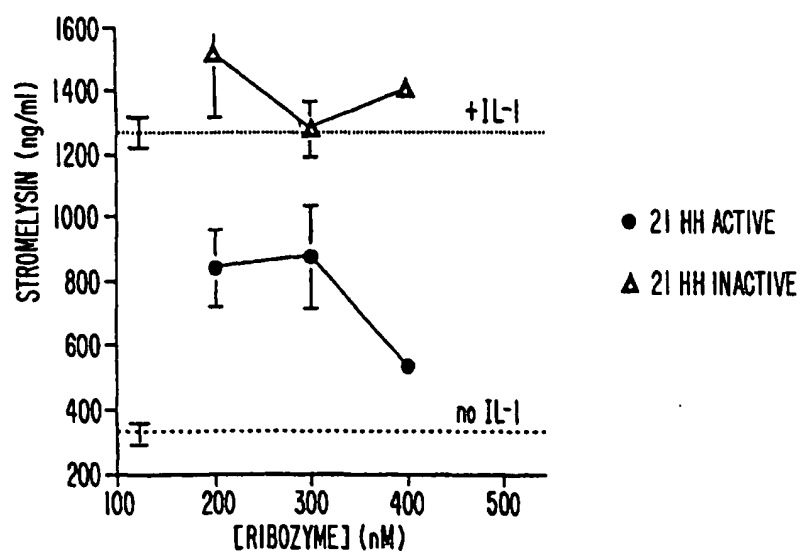


FIG. 8.

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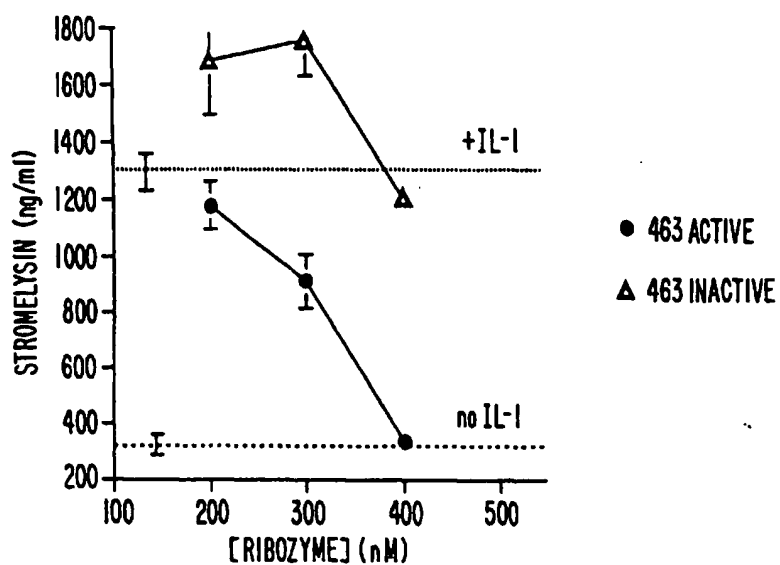


FIG. 9.

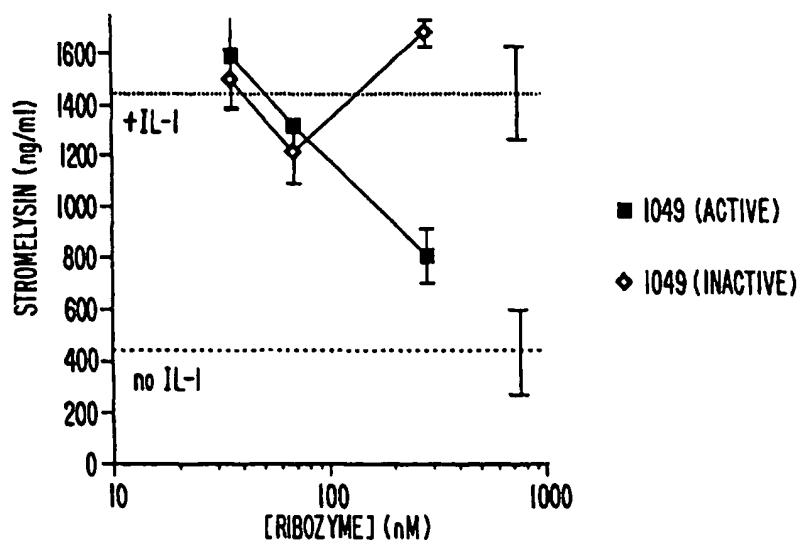


FIG. 10.

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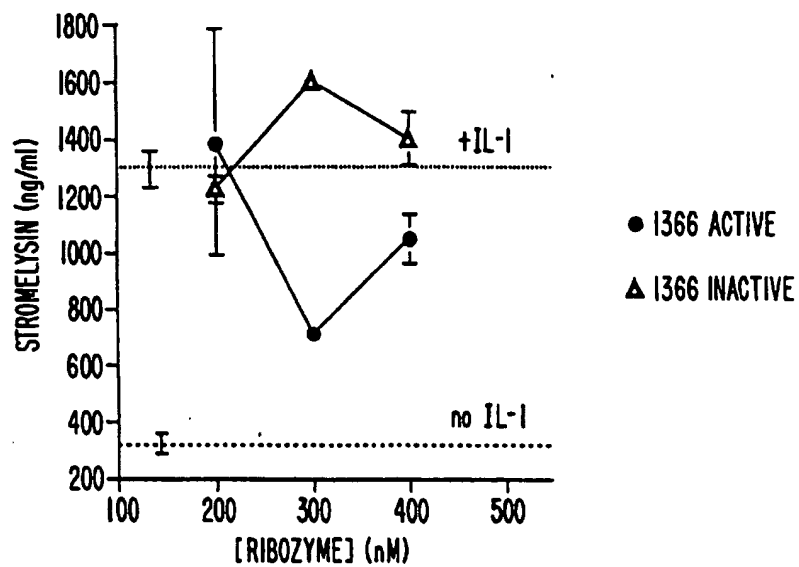


FIG. 11.

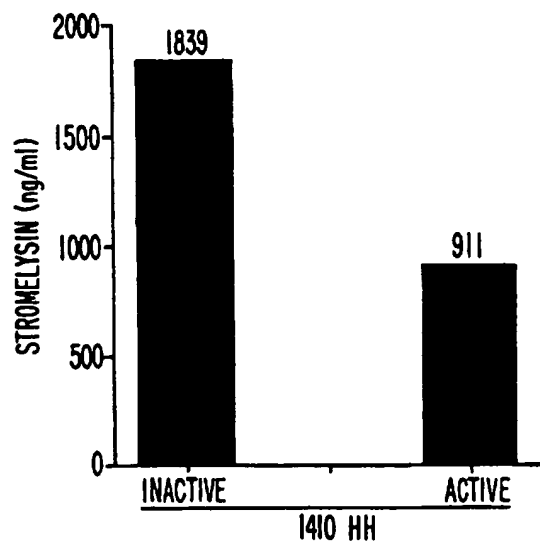


FIG. 12.

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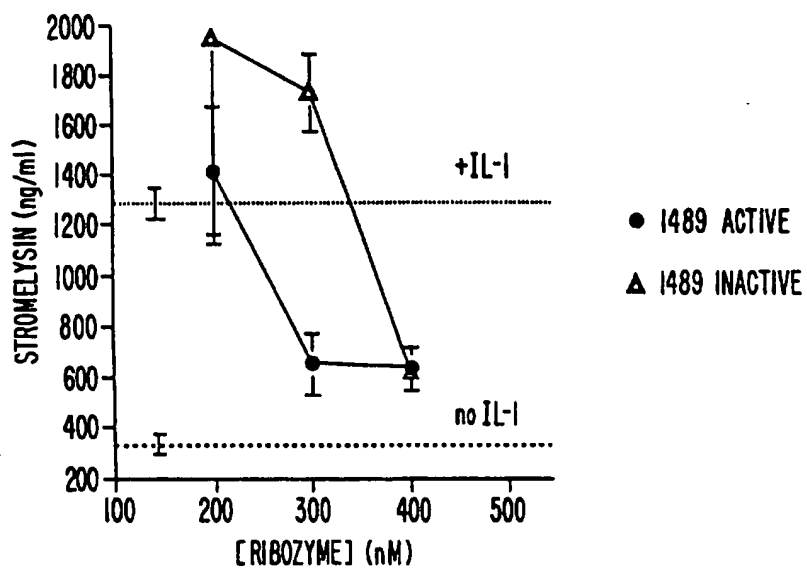


FIG. 13.

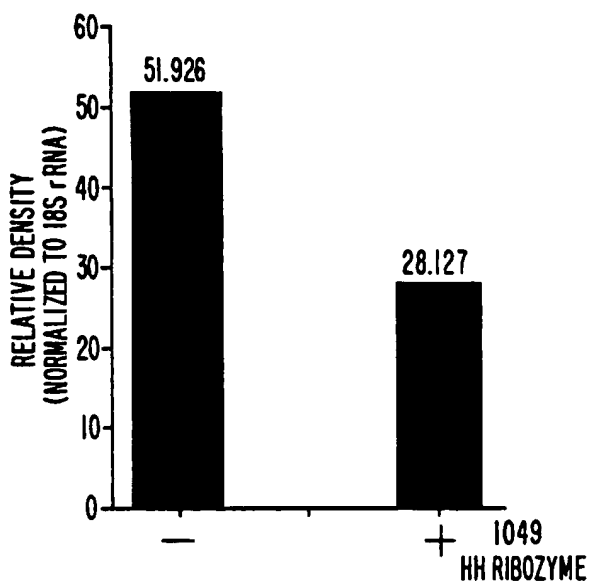


FIG. 14.

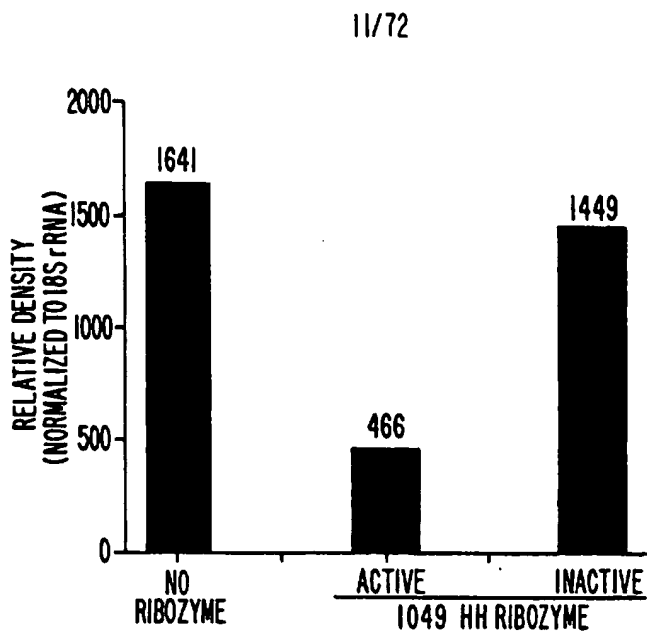


FIG. 15.

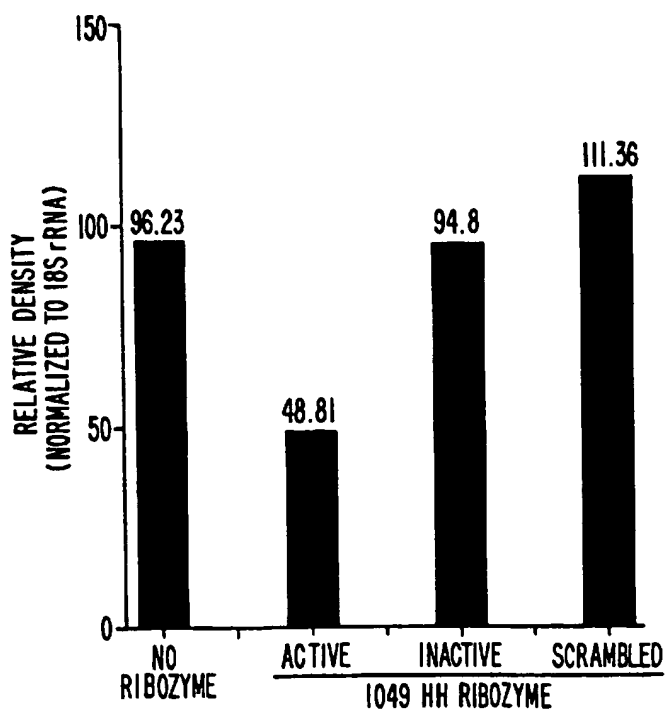
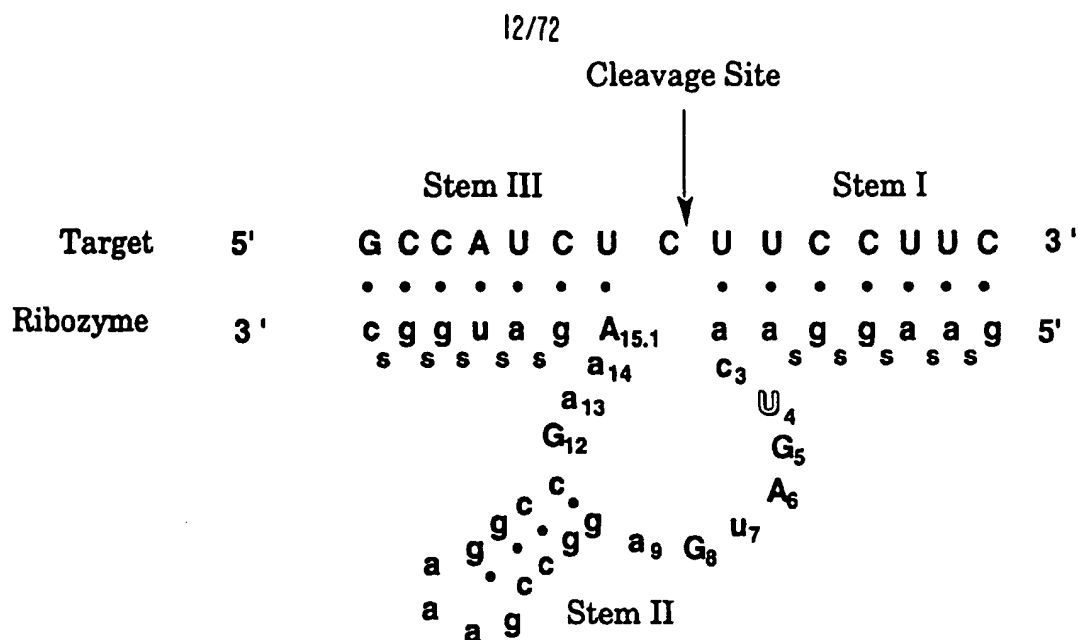


FIG. 16.



Upper case= ribonucleotides

Lower case= 2'-O-methyl nucleotides

U = 2'-C-Allyl modification

s = phosphorothioate linkages

FIG. 17a.

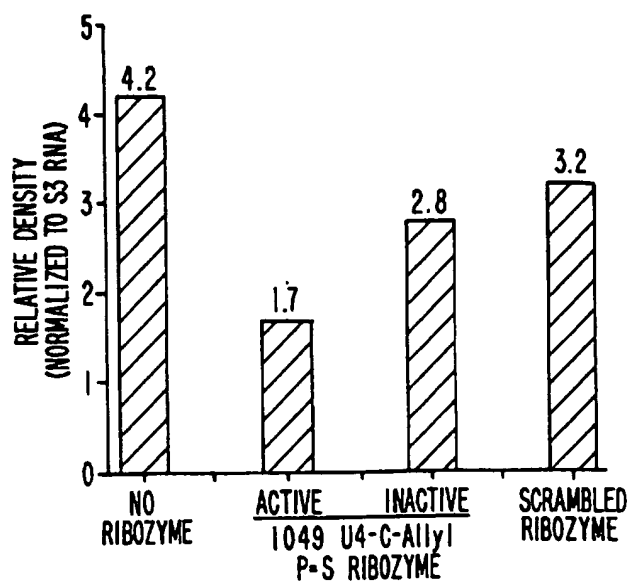


FIG. 17b.

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5'--- G C C A U C U C U U C C U U C --- 3'

3- π -c g g u a g A a a g g a a g 5'

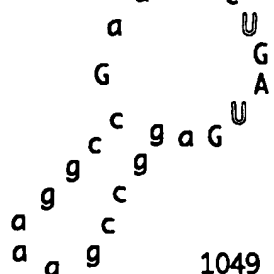


FIG. 18a.

UPPER CASE=ribonucleotides

LOWER CASE=2'-O-Methylnucleotides

U=2'-amino

s=phosphorothioatelinkages

1049 2'-AMINO P=S RIBOZYME

5'--- A U G C U G U U U U U G A A G --- 3'

3- π -u a c g a c A a a a c u u c 5'

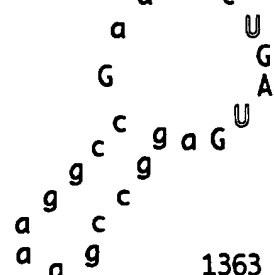


FIG. 18b.

UPPER CASE=ribonucleotides

LOWER CASE=2'-O-Methylnucleotides

U=2'-amino

s=phosphorothioatelinkages

1363 2'-AMINO P=S RIBOZYME

5'--- C U G U U U U U G A A G A A U --- 3'

3- π -g a c a a a A c u u c u u a 5'

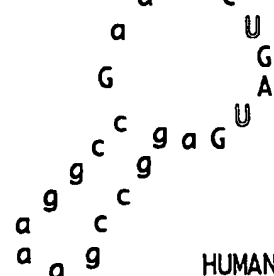


FIG. 18c.

UPPER CASE=ribonucleotides

LOWER CASE=2'-O-Methylnucleotides

U=2'-amino

s=phosphorothioatelinkages

HUMAN 1366 2'-AMINO P=S RIBOZYME

5'--- C U G U U U U U G A A G C A U --- 3'

3- π -g a c a a a A c u u c g u a 5'

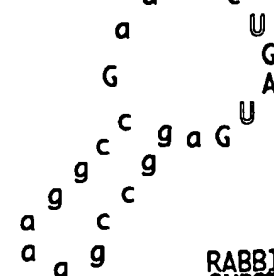


FIG. 18d.

UPPER CASE=ribonucleotides

LOWER CASE=2'-O-Methylnucleotides

U=2'-amino

s=phosphorothioatelinkages

RABBIT 1366 2'-AMINO P=S RIBOZYME

SUBSTITUTE SHEET (RULE 26)

FIG. 19.

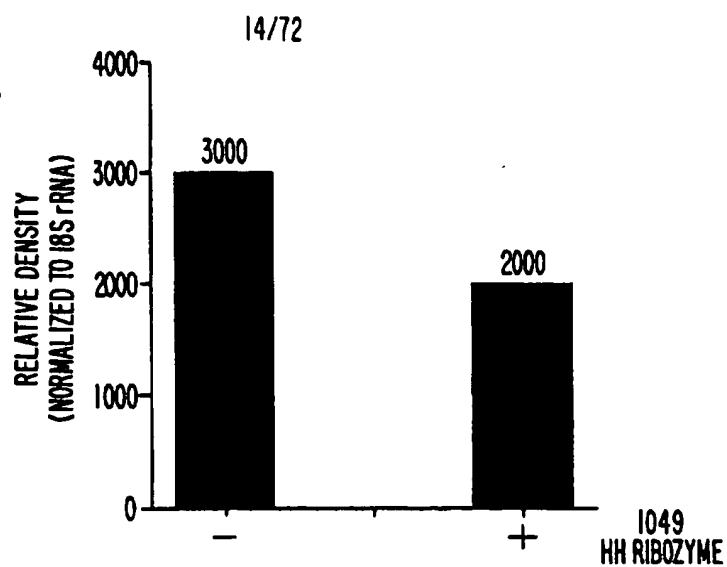


FIG. 20.

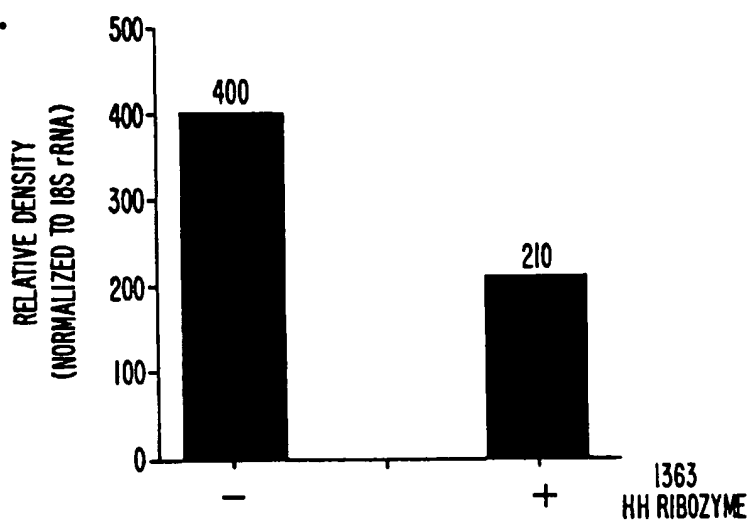
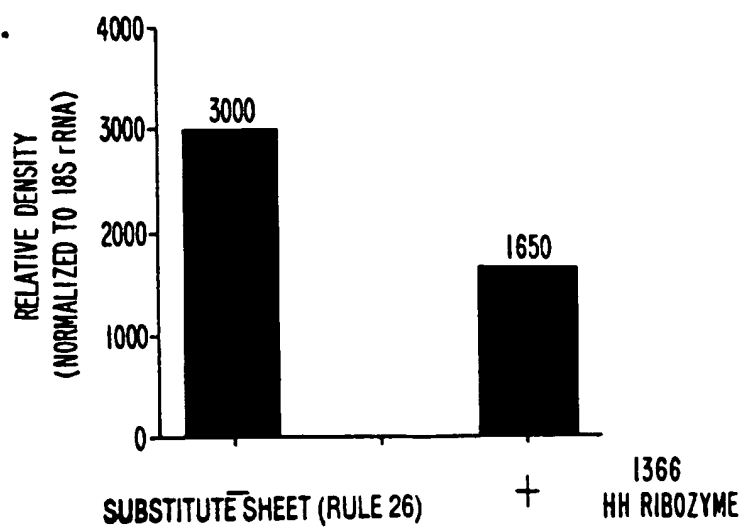
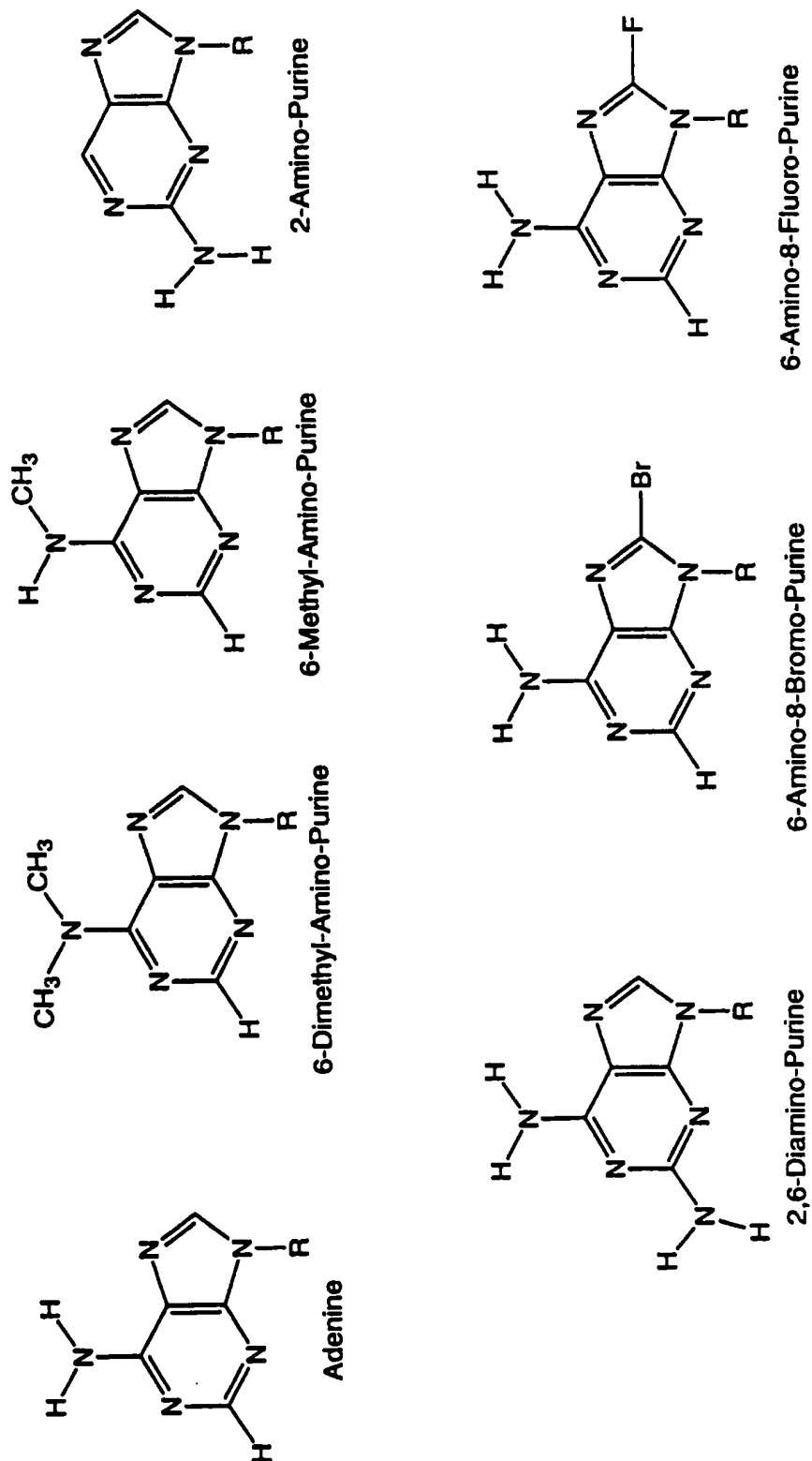


FIG. 21.



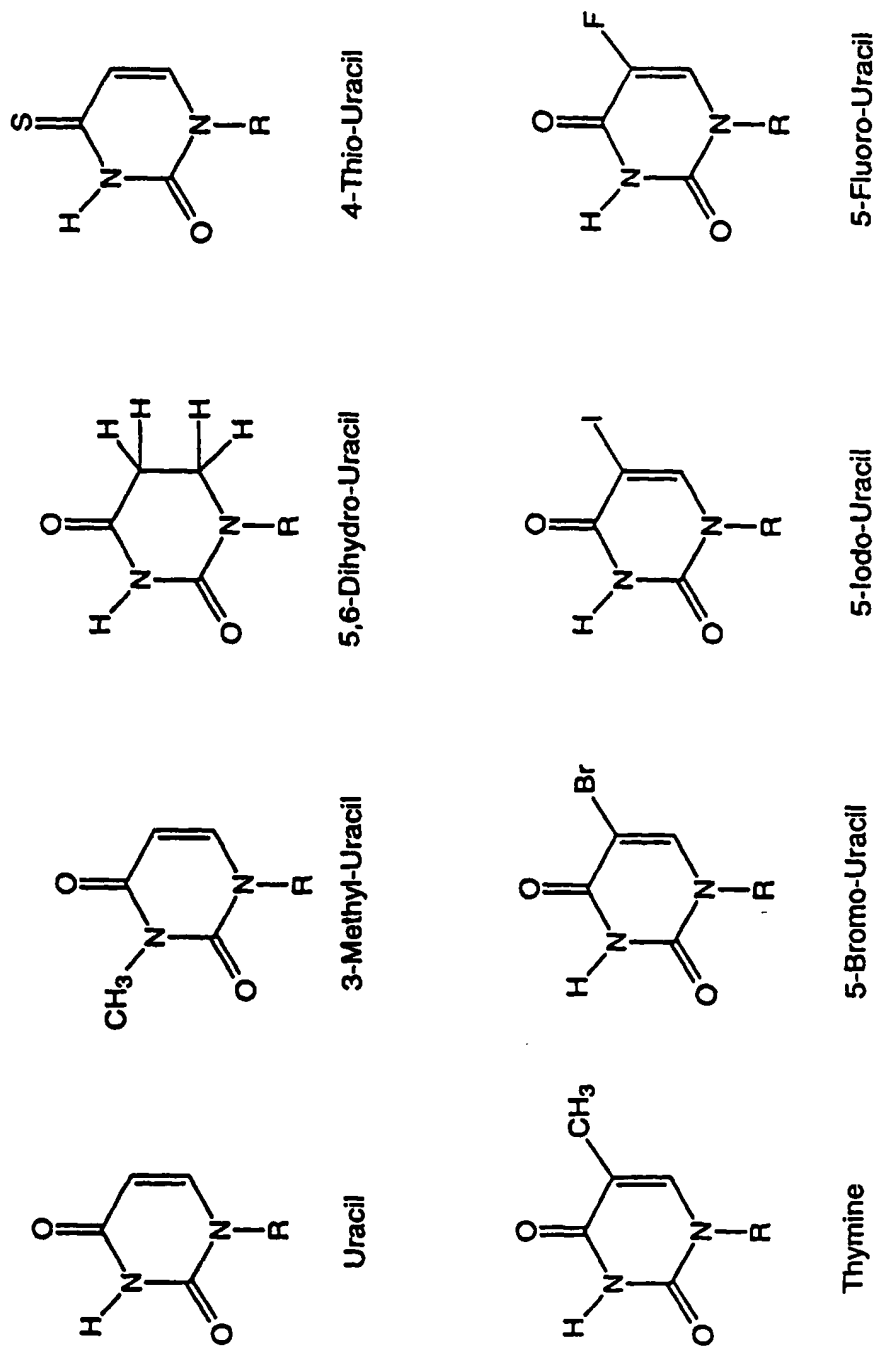
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FIG. 22a.



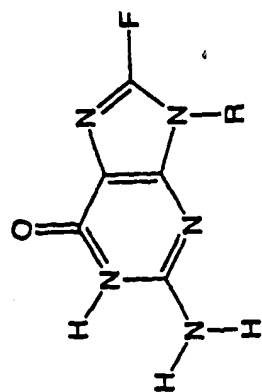
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FIG. 22b.

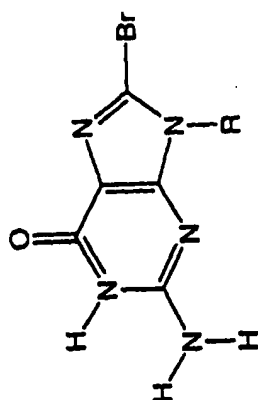


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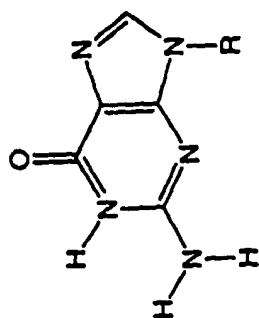
FIG. 22c.



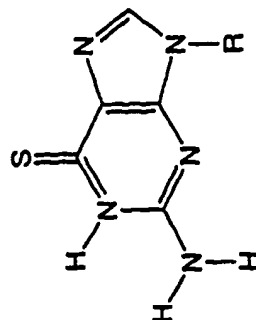
8-Fluoro-Guanine



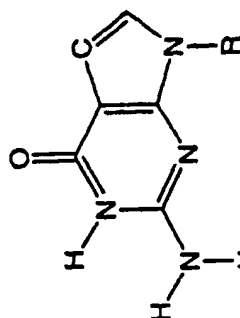
8-Bromo-Guanine



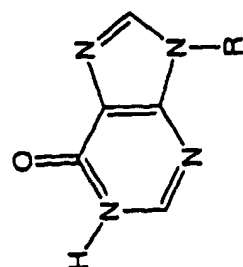
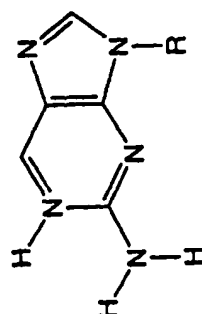
Guanine



6-Thio-Guanine



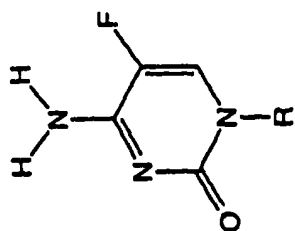
7-Deaza-Guanine

Hypoxanthine
(Inosine base)

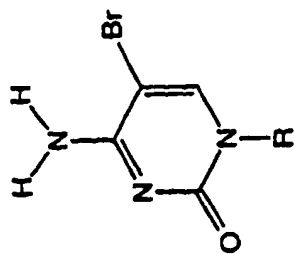
2-Amino-Purine

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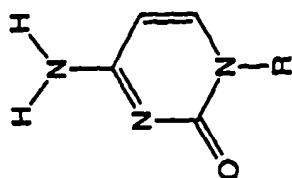
FIG. 22d.



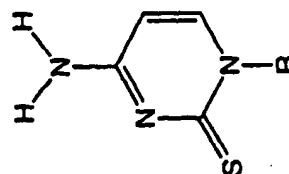
5-Fluoro-Cytosine



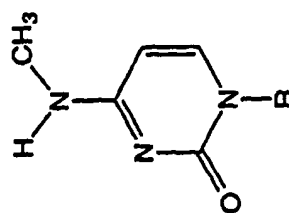
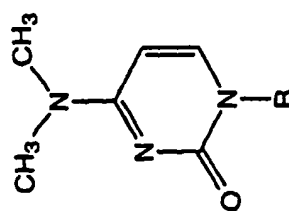
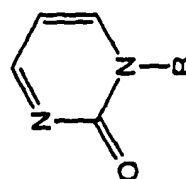
5-Bromo-Cytosine



Cytosine

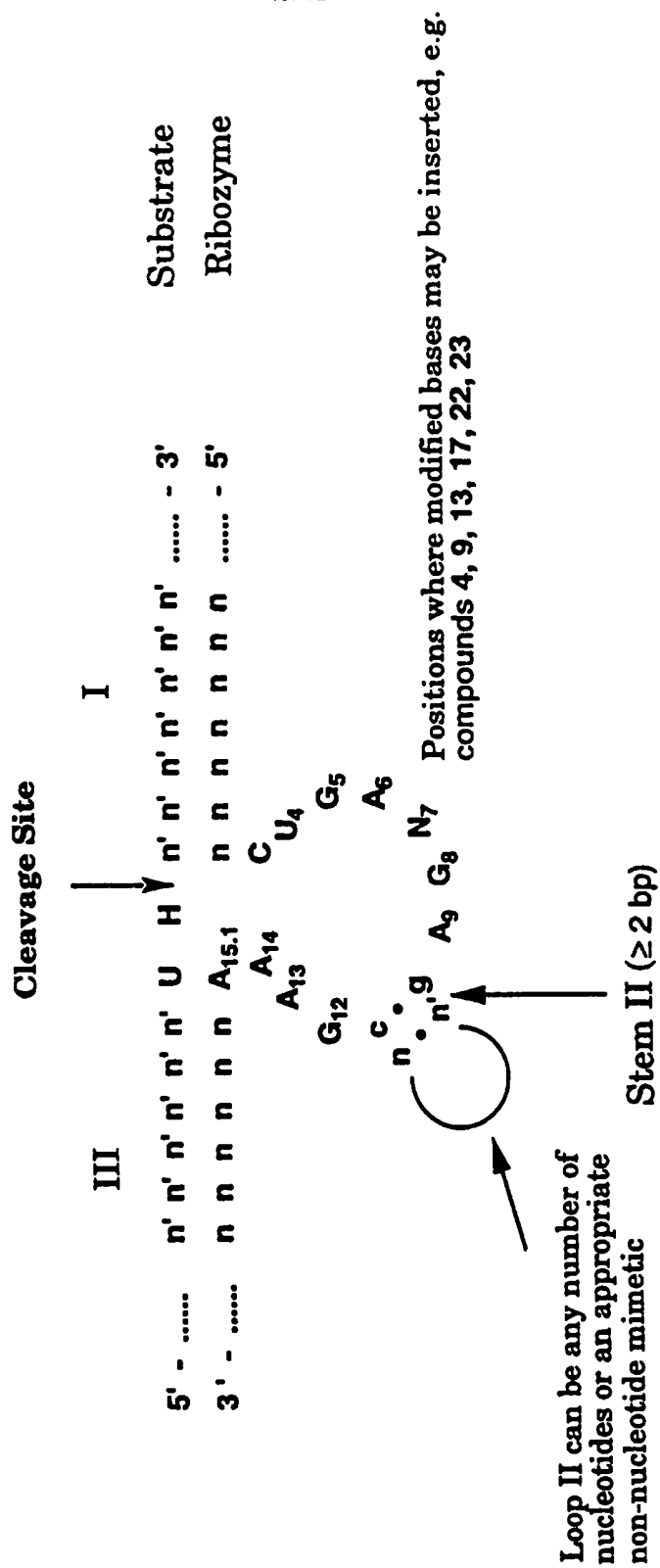


2-Thio-Cytosine

N⁴-Methyl-CytosineN⁴,N⁴-dimethyl-Cytosine

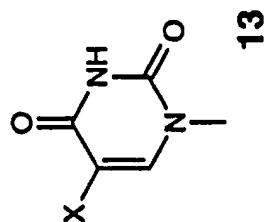
2-Pyridone

FIG. 23.

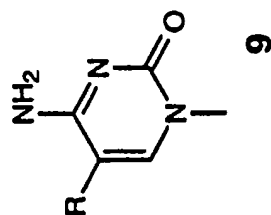


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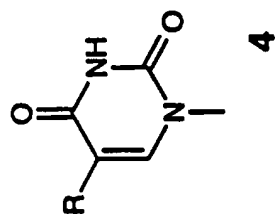
FIG. 24.



5-Halouridine

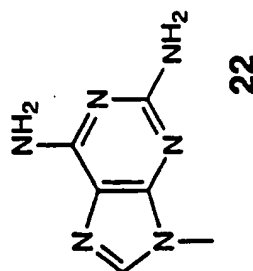
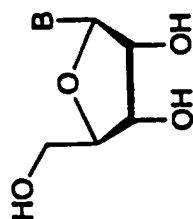


5-Alkylcytidine

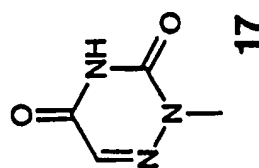


5-Alkyluridine

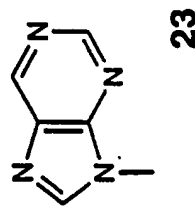
B =



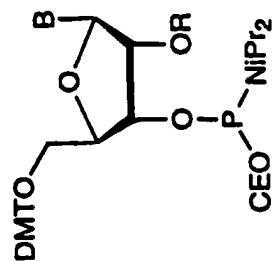
Diaminopurine



6-Azauridine

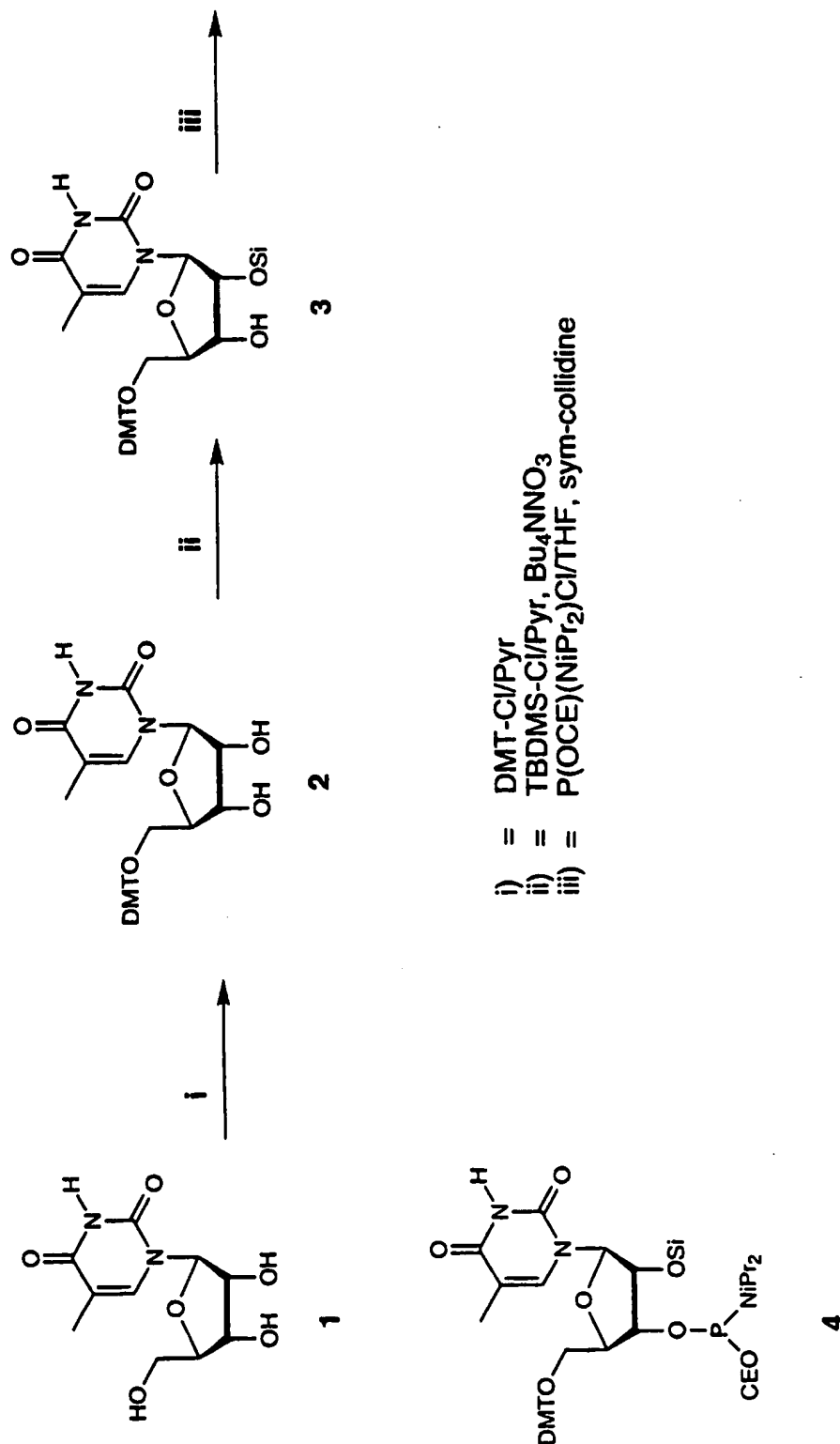


Purine



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FIG. 25.



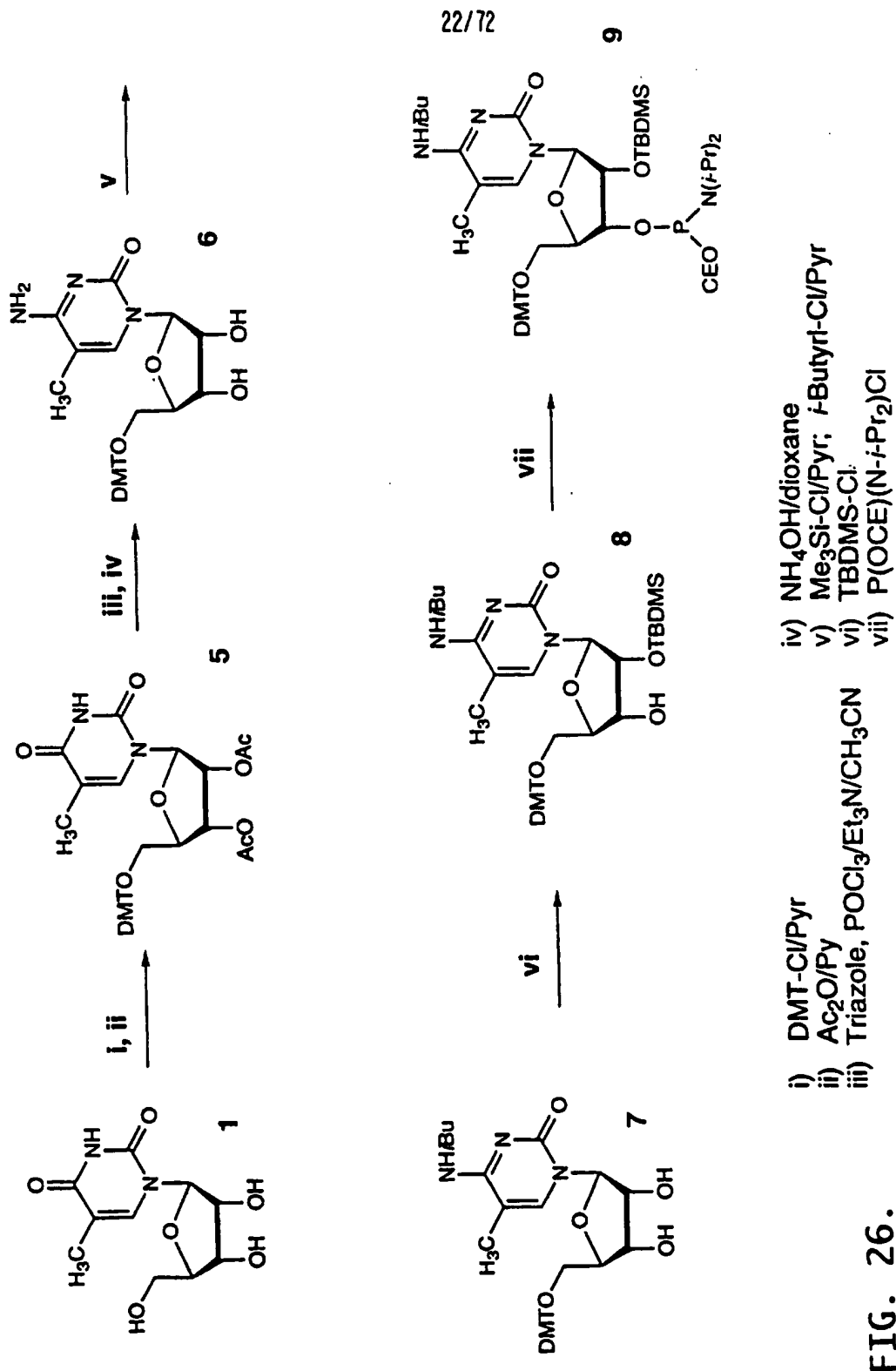
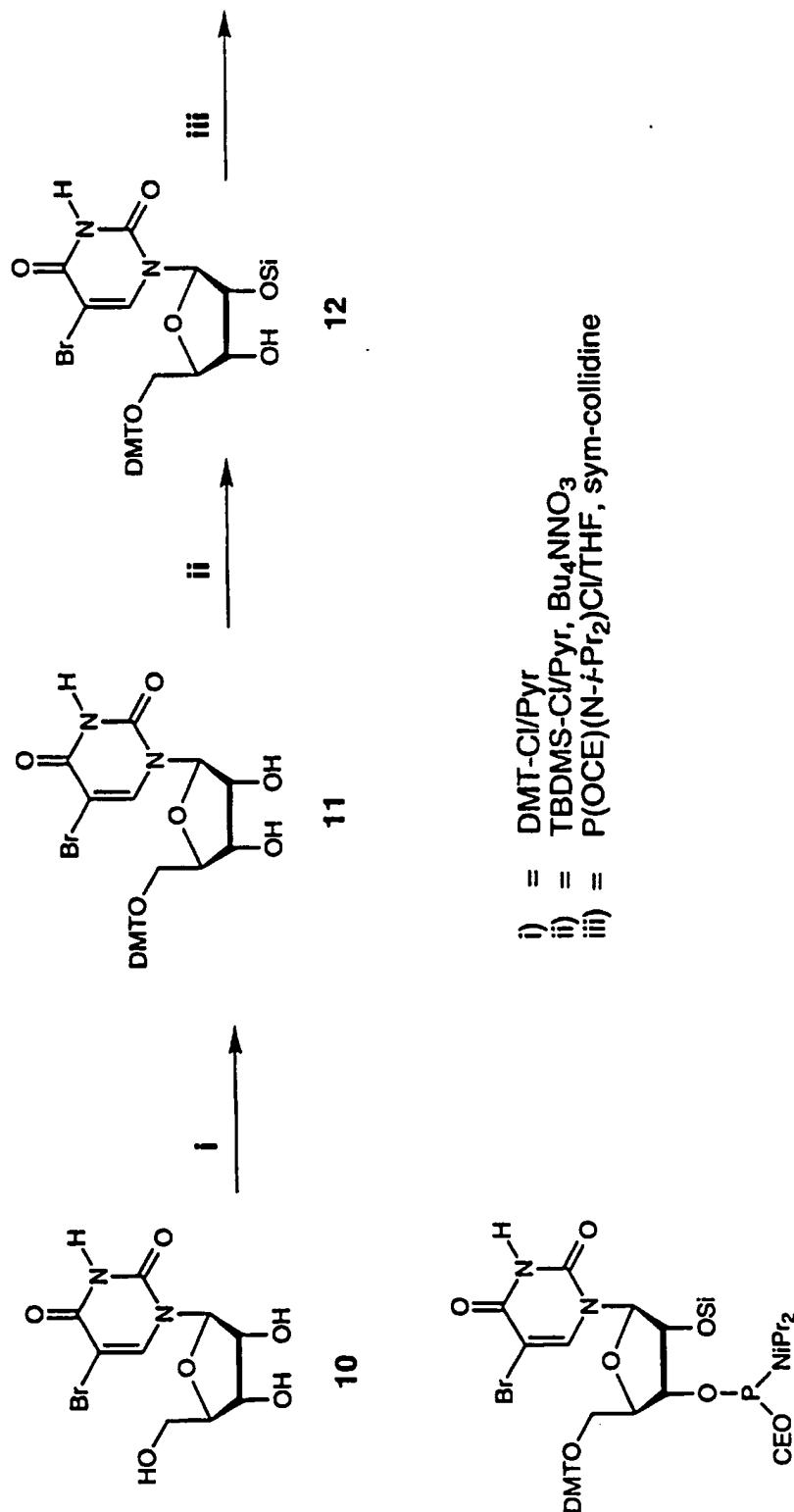


FIG. 26.

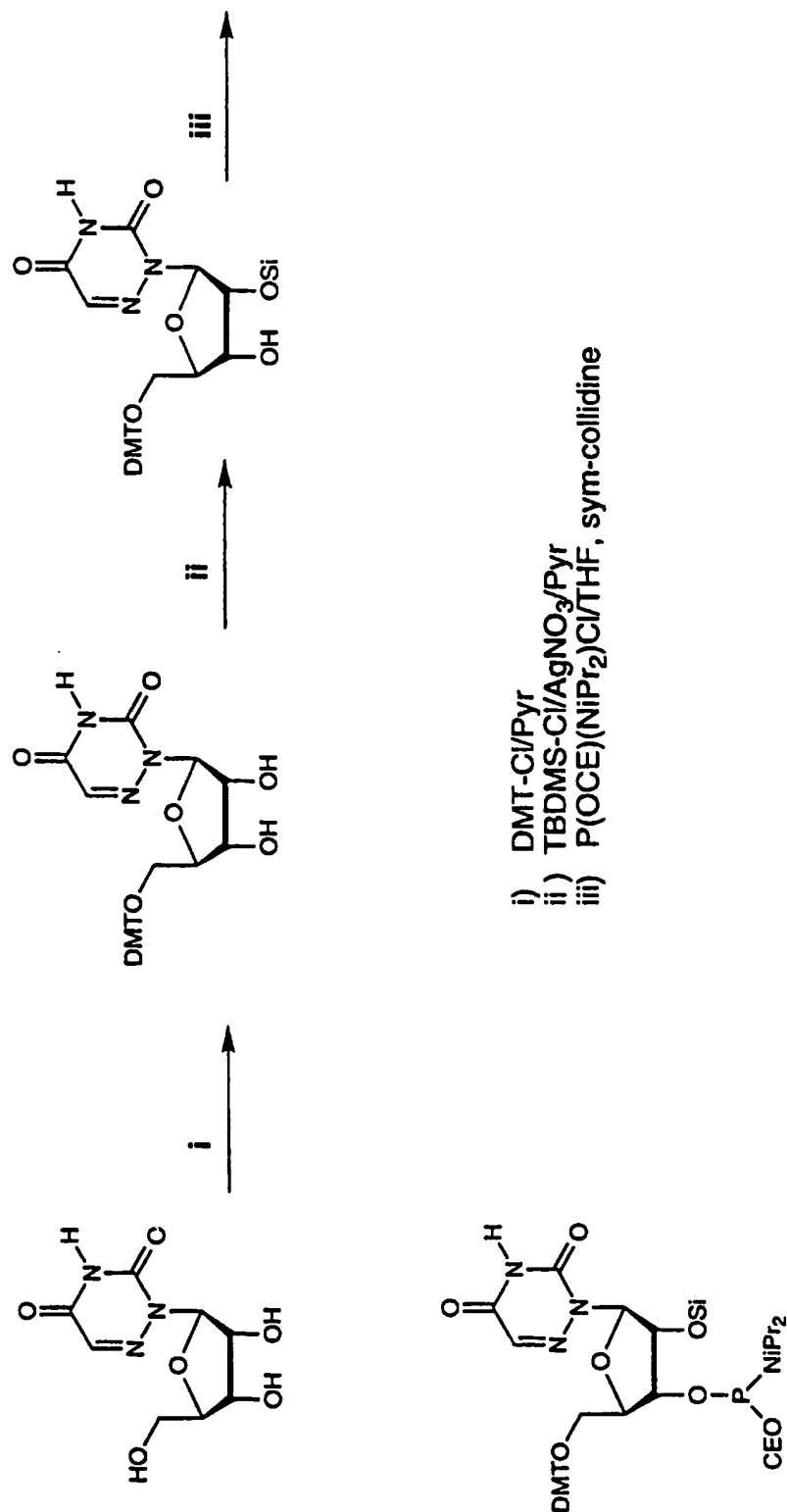
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FIG. 27.

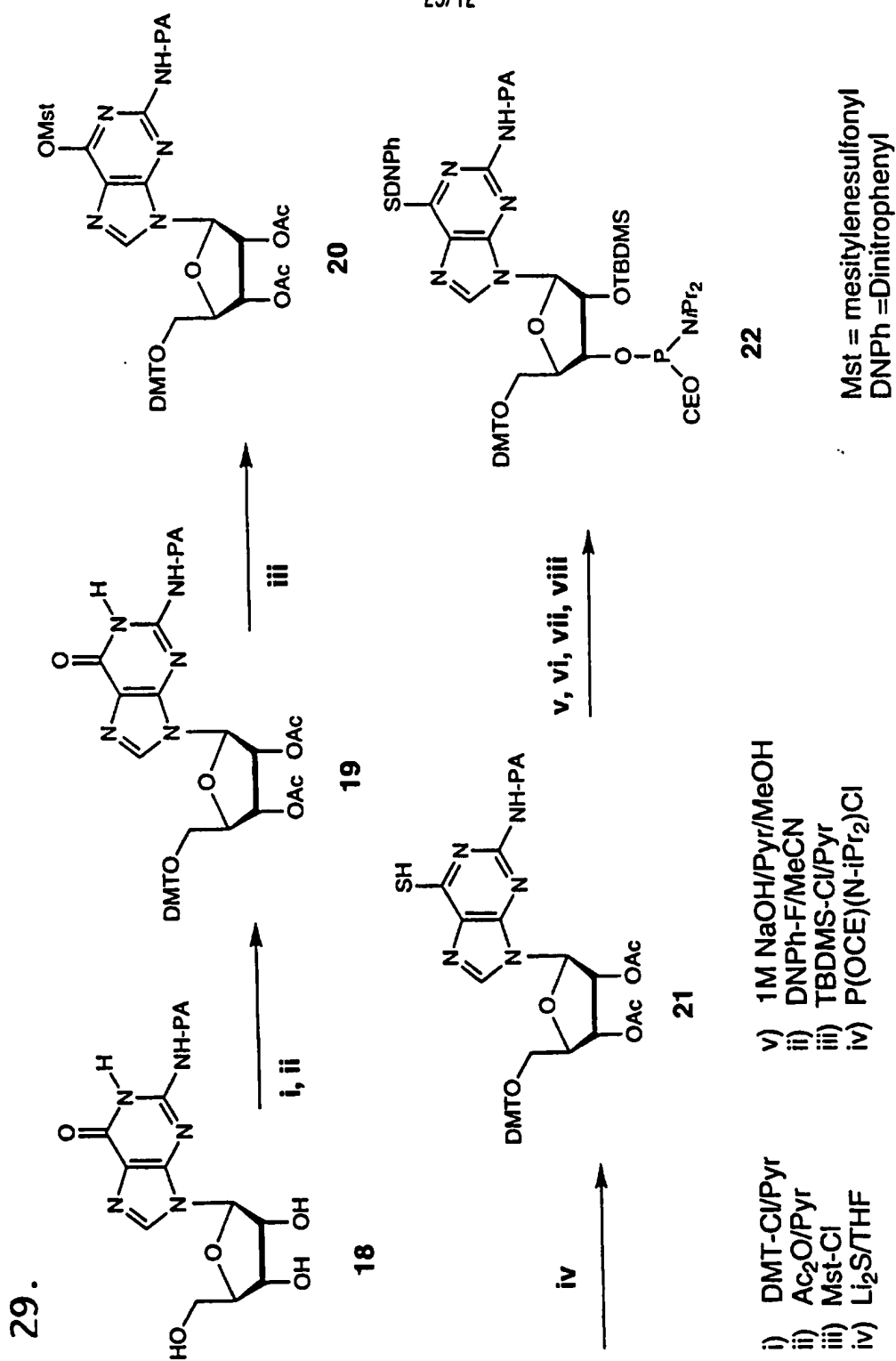


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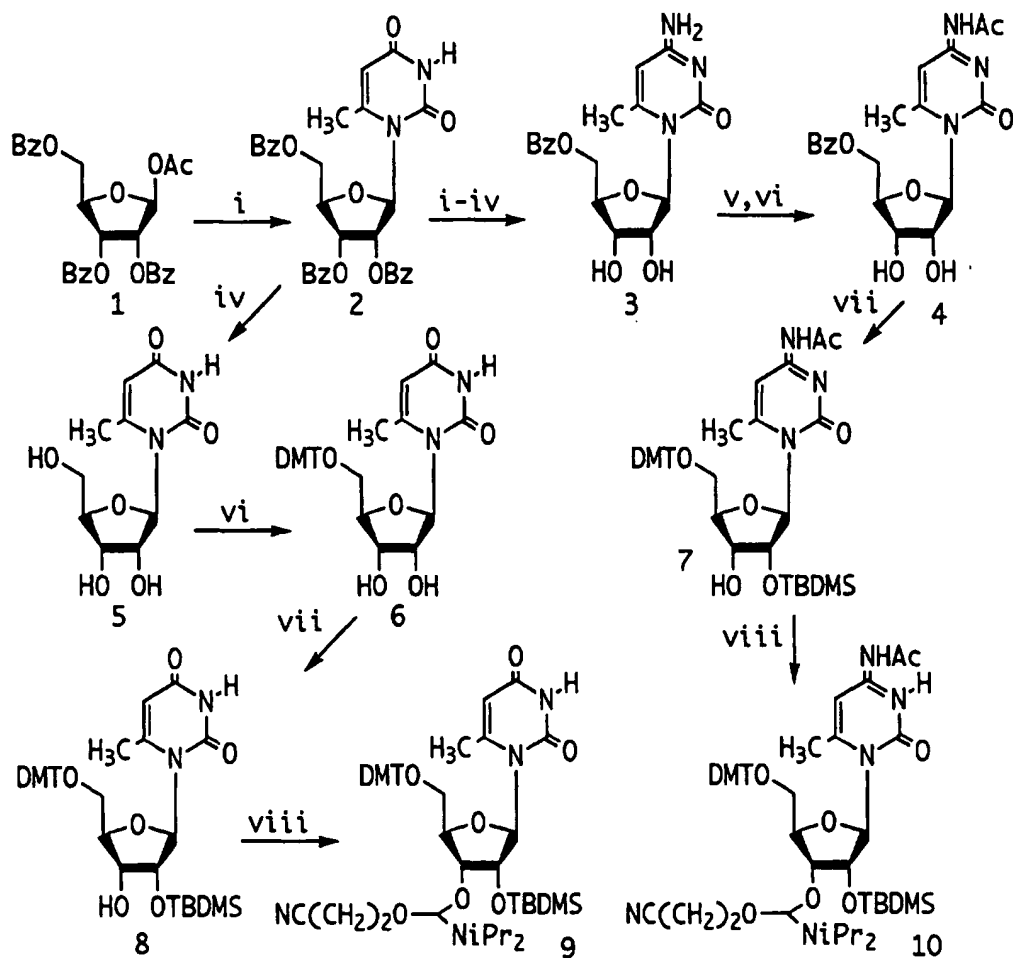
FIG. 28.



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REAGENTS AND CONDITIONS:

- i) 6-Me-Ura^{TMS}, CF₃SO₃SIME₃, 0°C;
- ii) 1,2,4-triazole, POCl₃; iii) NH₄OH/dioxane;
- iv) 2M NaOH/Pyr/MeOH; v) MeSI-CL/PYR, THEN AC₂O;
- vi) DMT-CL/Pyr;
- vii) TBDMS-CL/AgNO₃/Pyr/THF;
- viii) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, DIPEA/CH₂Cl₂.

FIG. 30.

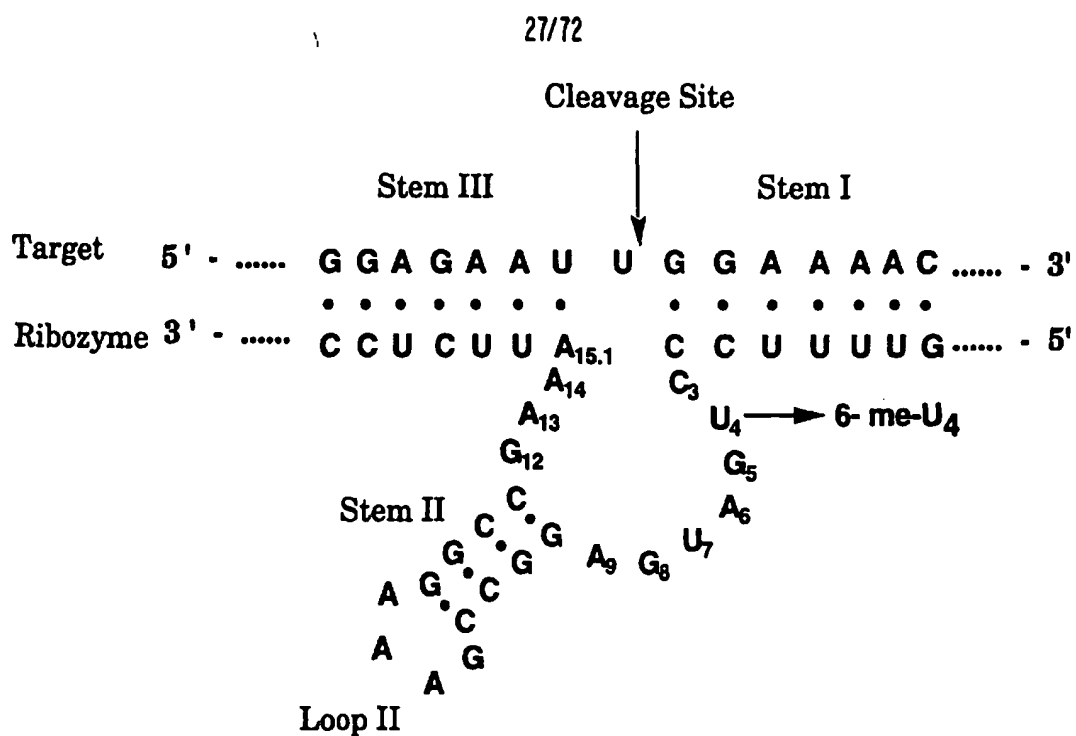
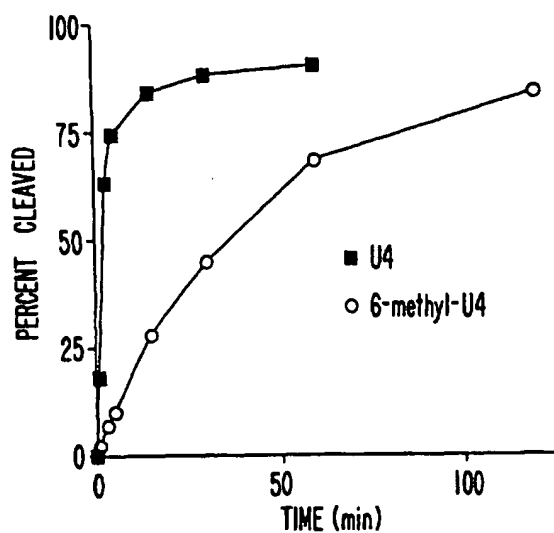


FIG. 31.



[Ribozyme]=40nM [Substrate]=~1nM

FIG. 32.

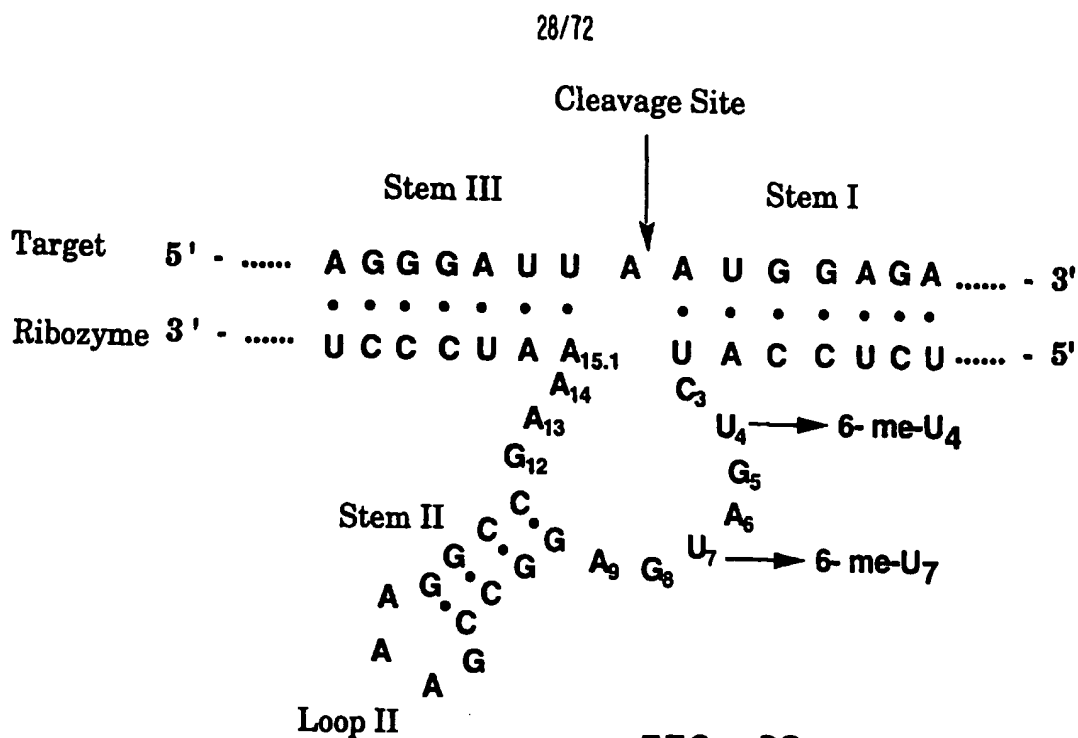
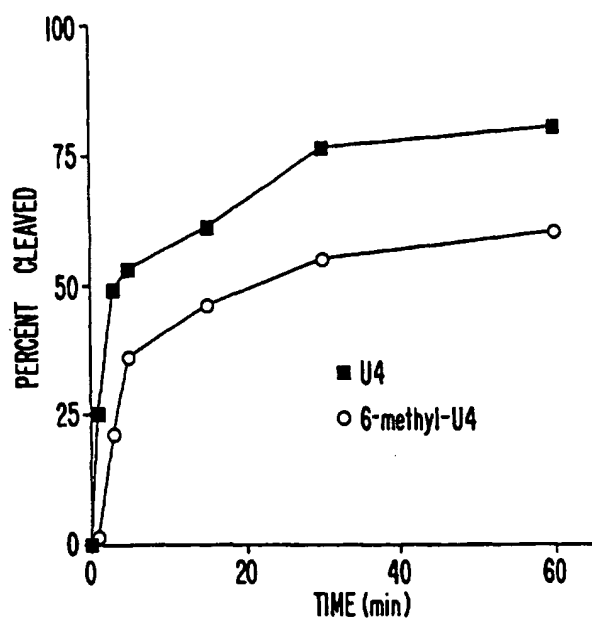


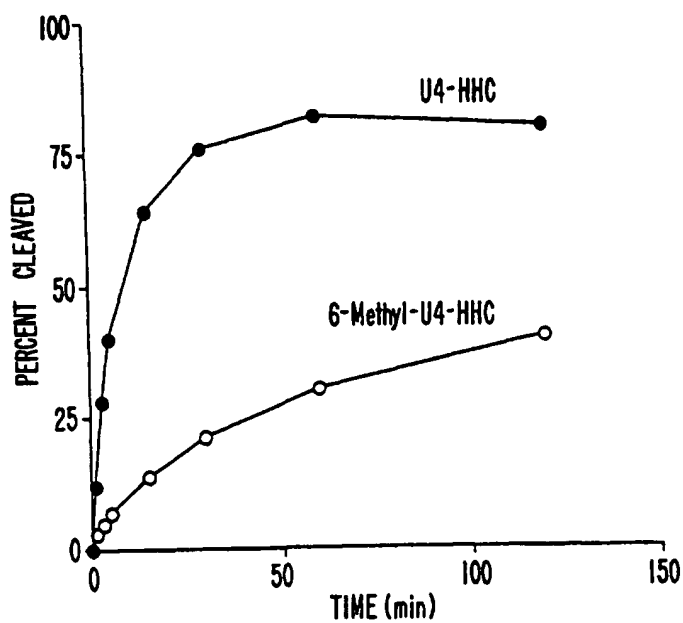
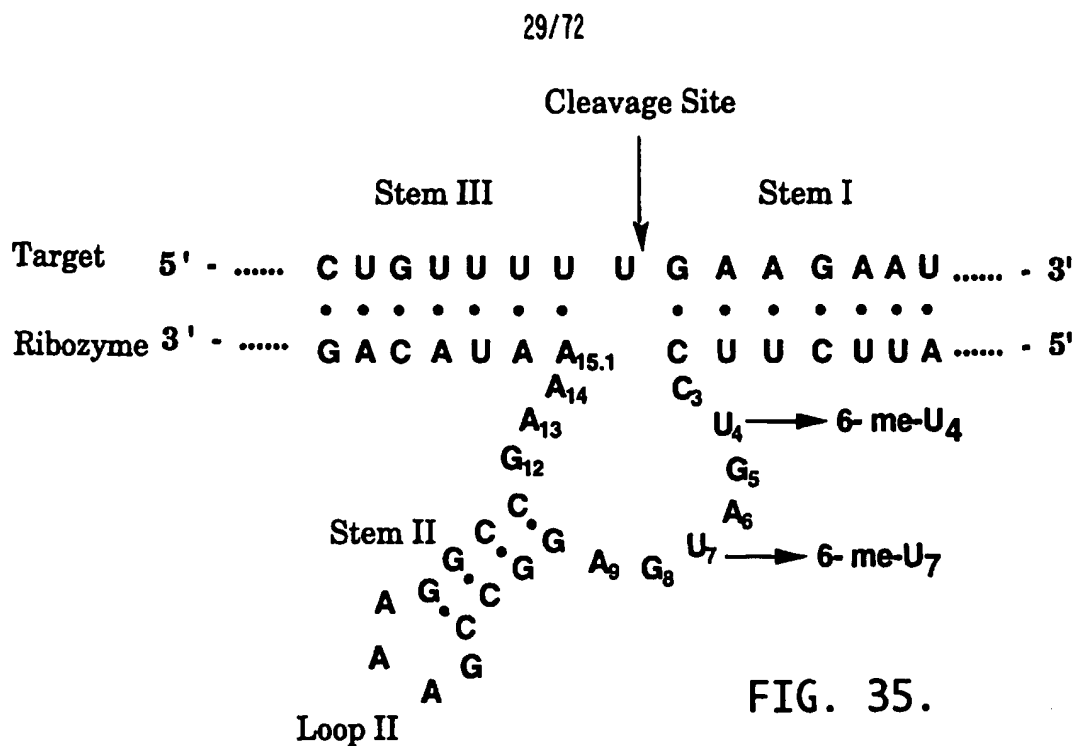
FIG. 33.



[Ribozyme]=40nM [Substrate]=~1nM

FIG. 34.

SUBSTITUTE SHEET (RULE 26)



[Ribozyme]=40nM [Substrate]=~1nM

FIG. 36.
SUBSTITUTE SHEET (RULE 26)

FIG. 37.

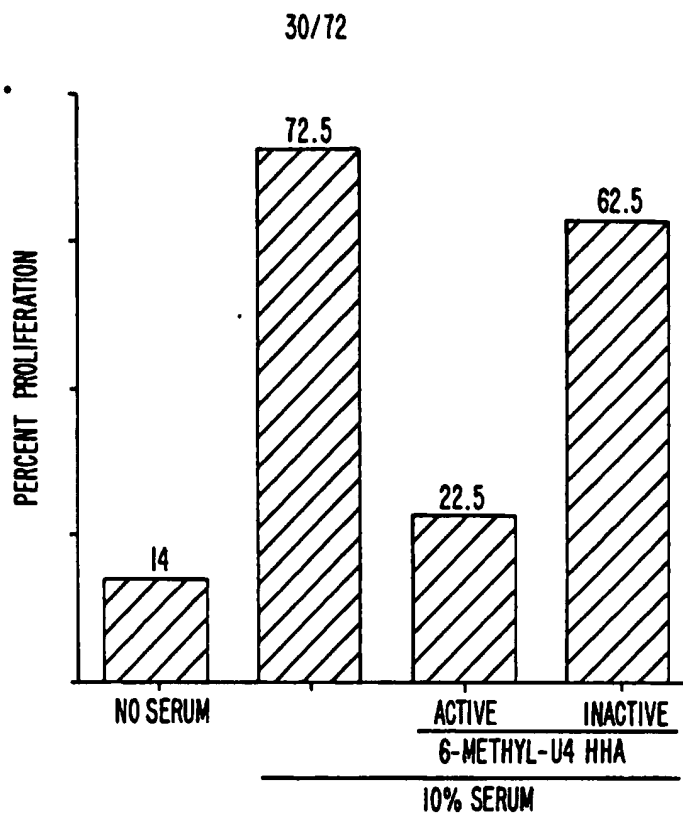
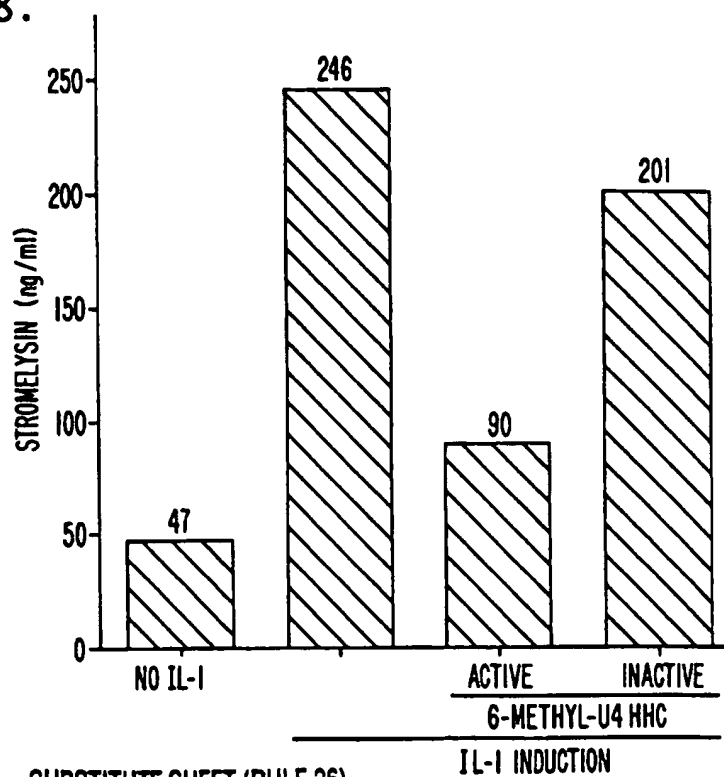
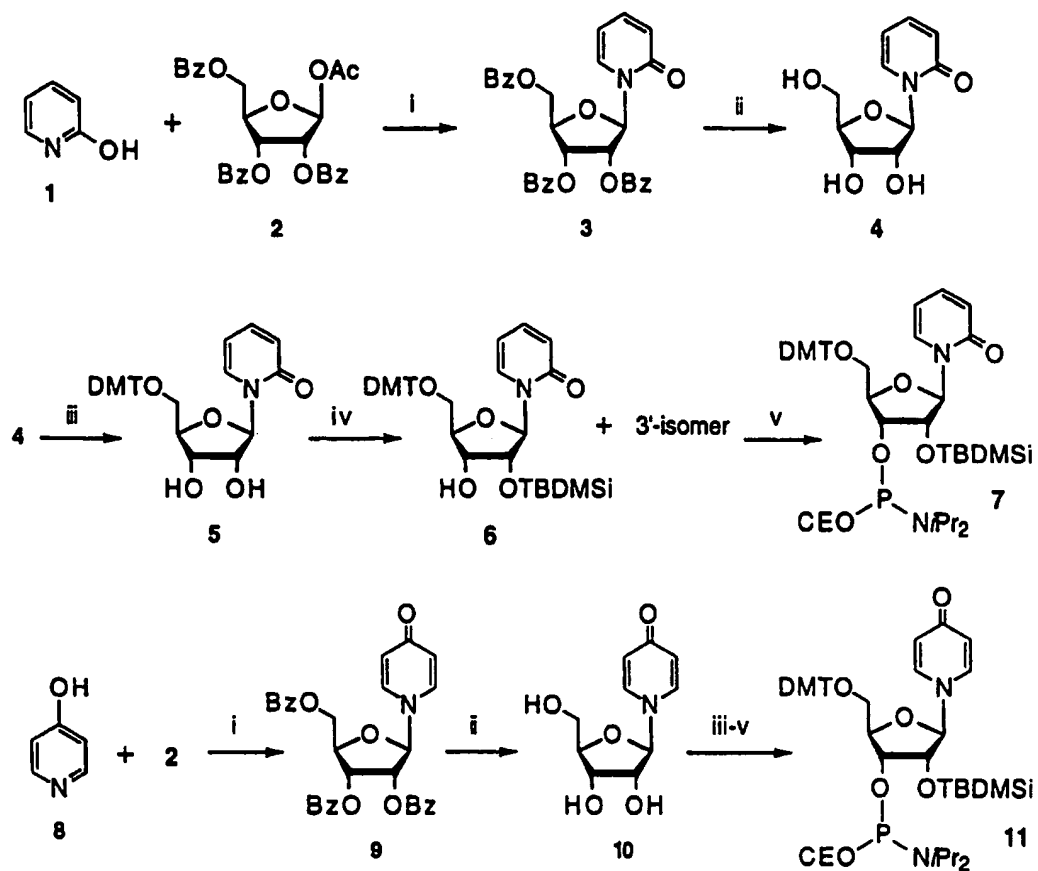


FIG. 38.



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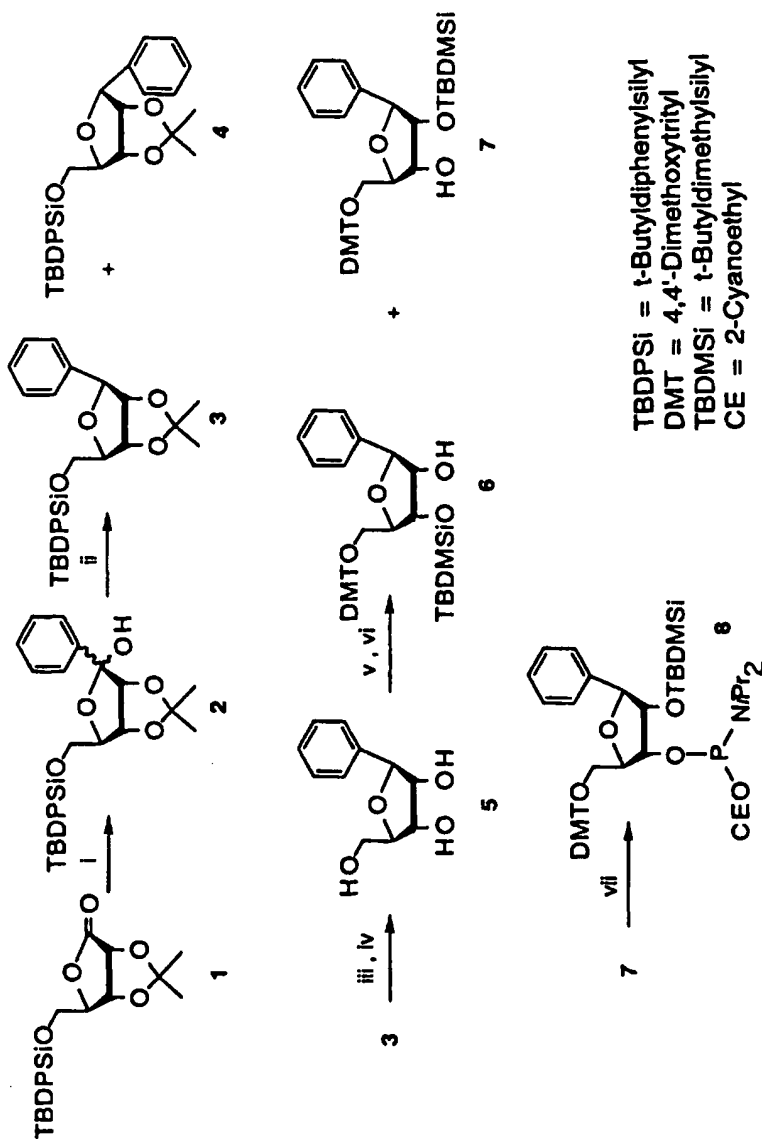


Bz = Benzoyl
 Ac = Acetyl
 DMT = 4,4'-Dimethoxytrityl
 TBDMSi = *t*-Butyldimethylsilyl
 CE = 2-cyanoethyl

Reagents and Conditions: i: N,O-bis(trimethylsilyl)acetamide (BSA)/TMSTf/
 CH₃CN, 70 °C, ii: NaOCH₃/CH₃OH, iii: DMT-Cl/DMAP/Et₃N/Pyr, iv: TBDMSi-
 Cl/AgNO₃/Pyr/THF, v: P(OCE)(N-*i*Pr₂)Cl/DIPEA/1-MeIm/CH₂Cl₂.

FIG. 39.

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Reagents and Conditions: i: PhLi/THF, -78 °C, ii: Et₃SiH/BF₃·Et₂O/CH₃CN, -40 °C,
 iii: 1M TBAF/THF, iv: 70 % aq. CH₃COOH, 100 °C, v: DMT-C/DMAPI/Et₃N/Pyr, vi:
 TBDMSi-CI/AgNO₃/Pyr/THF, vii: P(OCE)(N-IPr₂)Cl/DIPEA/1-Melm/CH₂Cl₂.

FIG. 40.

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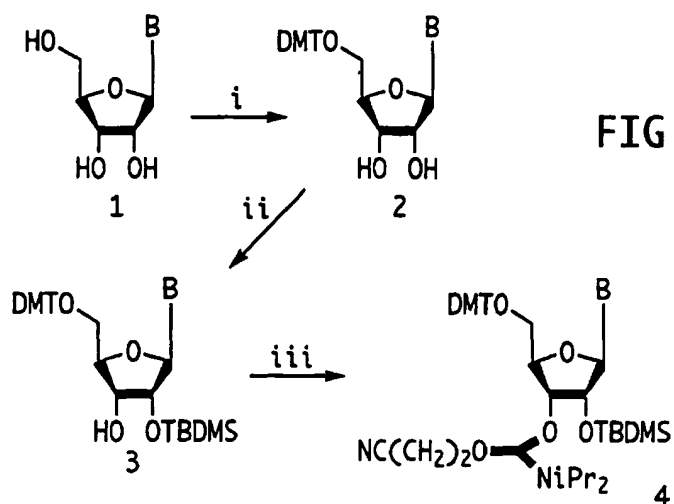


FIG. 41.

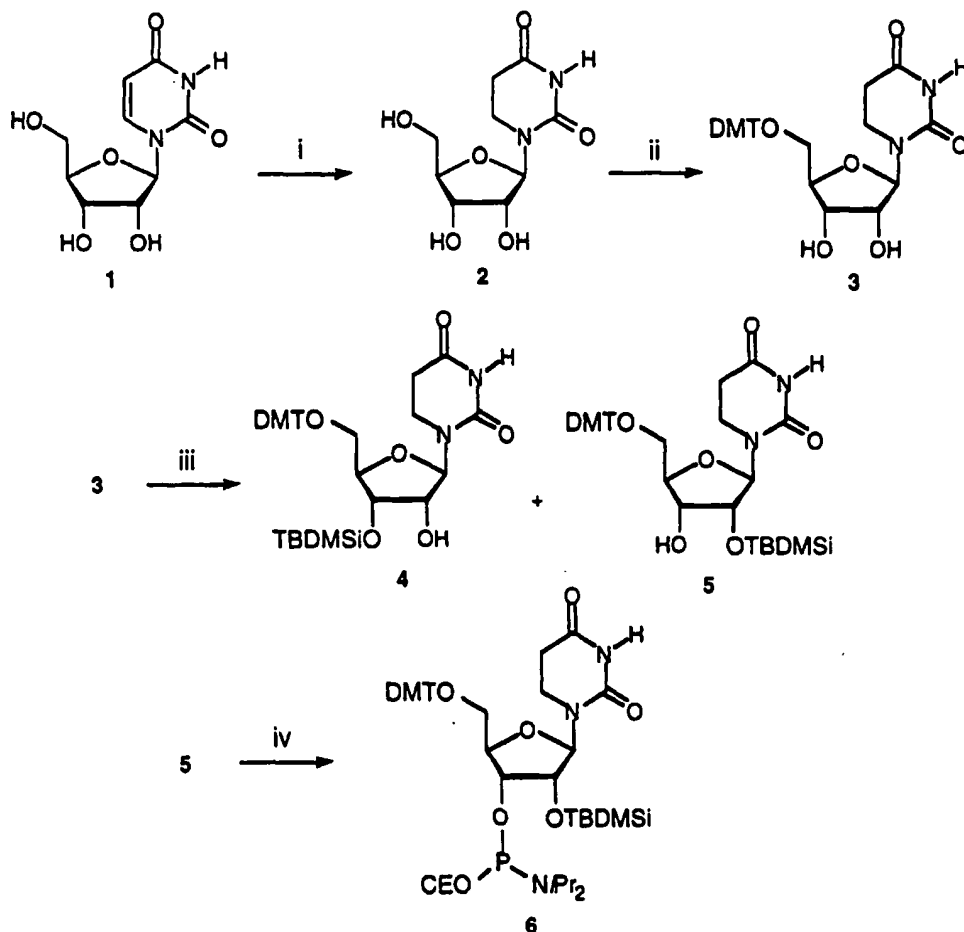
B=Pseudo U,2,4,6-trimethoxy benzene or 3-methyl U

REAGENTS AND CONDITIONS:

- i) DMT-Cl/Pyr;
- ii) TBDMS-Cl/AgNO₃/Pyr/THF;
- iii) 2-cyanoethyl-N,N-diisopropylchlorophosphoramidite, DIPEA/CH₂Cl₂.

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FIG. 42.



DMT = 4,4'-Dimethoxytrityl
 TBDMSi = t-Butyldimethylsilyl
 CE = 2-Cyanoethyl

Reagents and Conditions: i: Pd/Rh, H₂ 60 psi, ii: DMT-Cl/DMAP/Et₃N/Pyr,
 iii: TBDMSi-Cl/AgNO₃/Pyr/THF, iv: P(OCE)(N-iPr₂)Cl/DIPEA/1-MeIm/CH₂Cl₂

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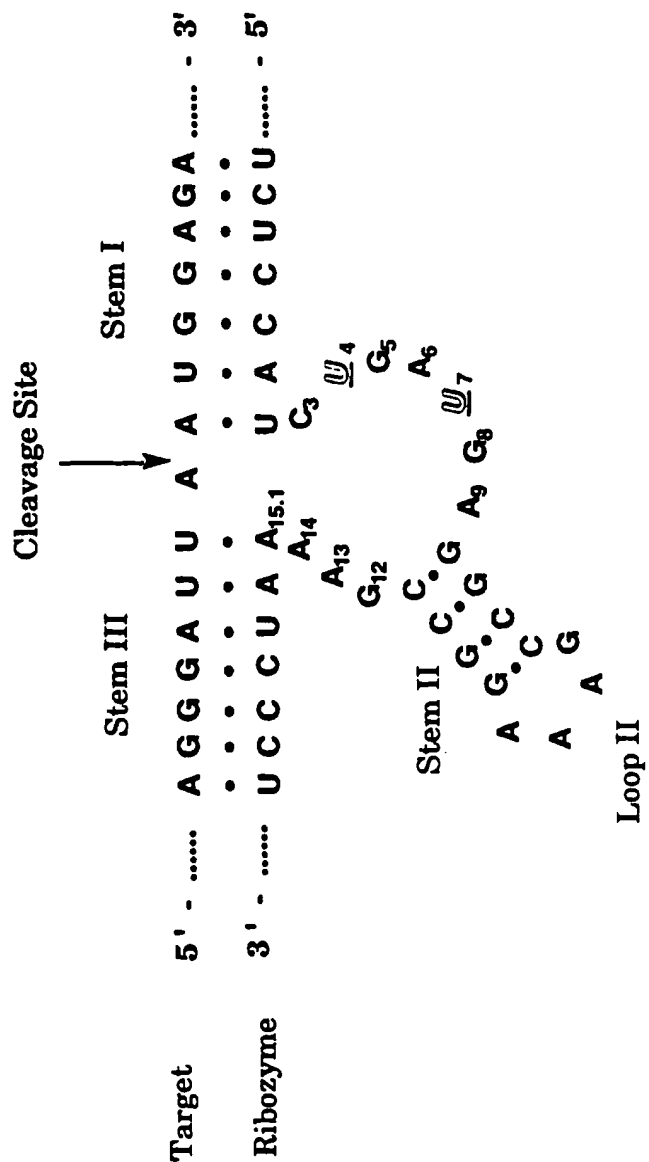
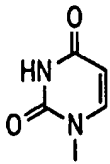
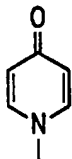
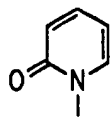

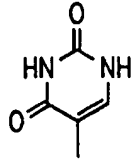
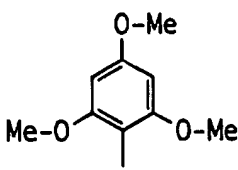
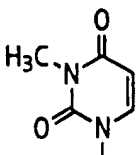


FIG. 43a.

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FIG. 43b.

BASE MODIFICATIONS		HH nt. POSITION	
		<u>4</u>	<u>7</u>
		<u>k_{obs}(min⁻¹)</u>	
	U	2.1	2.1
	Pyridin-4-one	0.04	≥10
	Pyridin-2-one	0.03	1.2
	Phenyl	0.05	2.5
	Pseudo U	1.0	0.22
	3-O-Methoxy Benzene	0.02	0.14
	3-Me thyl U	0.02	4.6

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FIG. 44a.

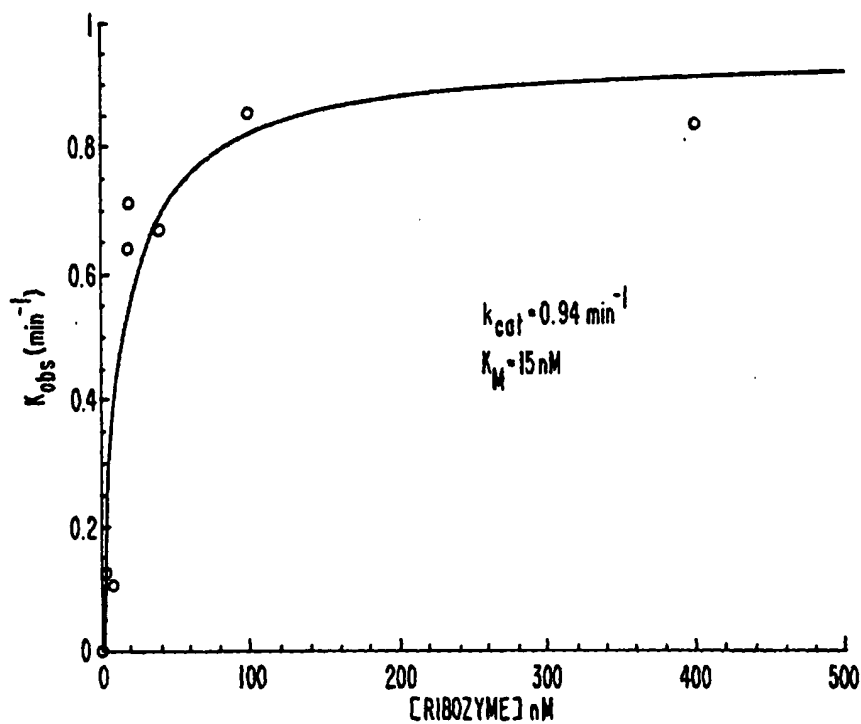
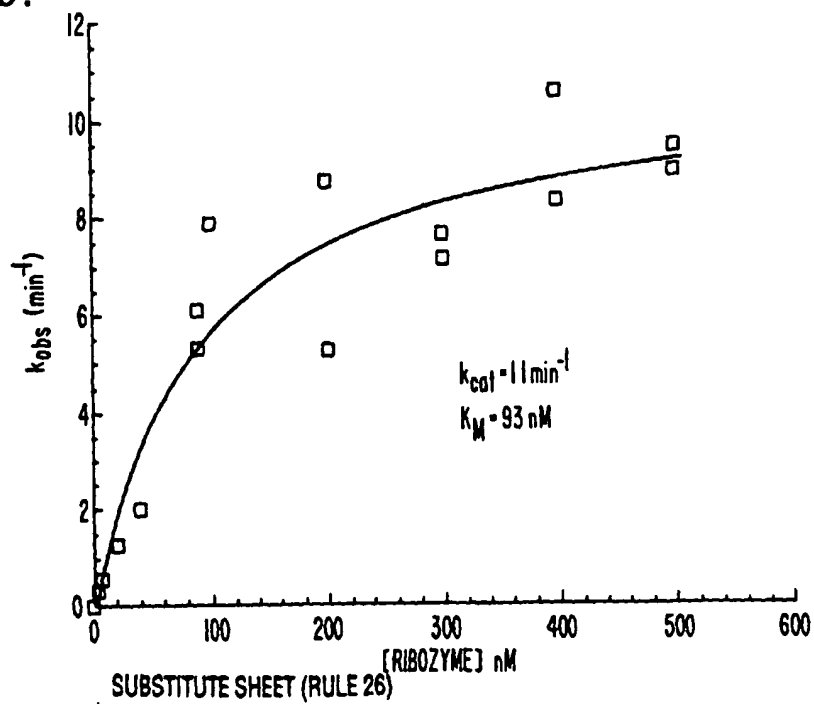


FIG. 44b.



SUBSTITUTE SHEET (RULE 26)

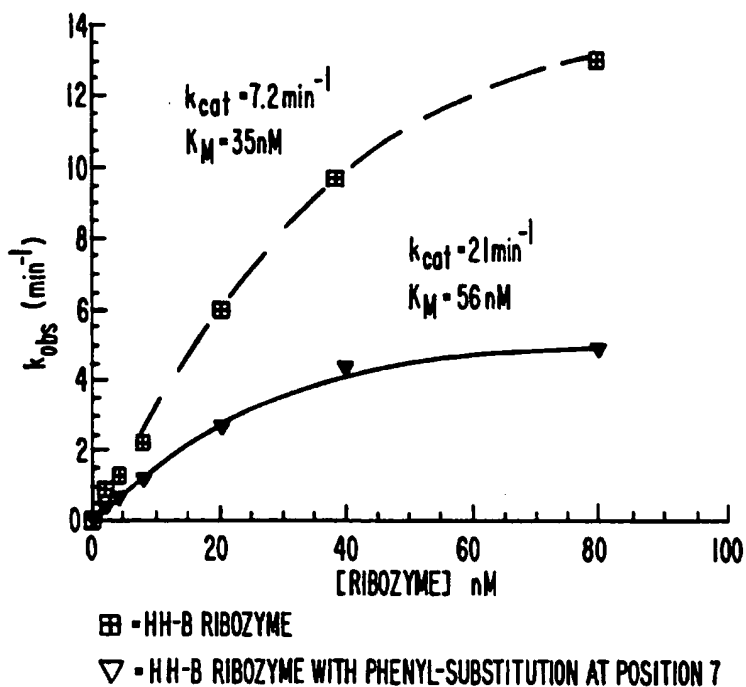
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FIG. 44c.

FIG. 45.

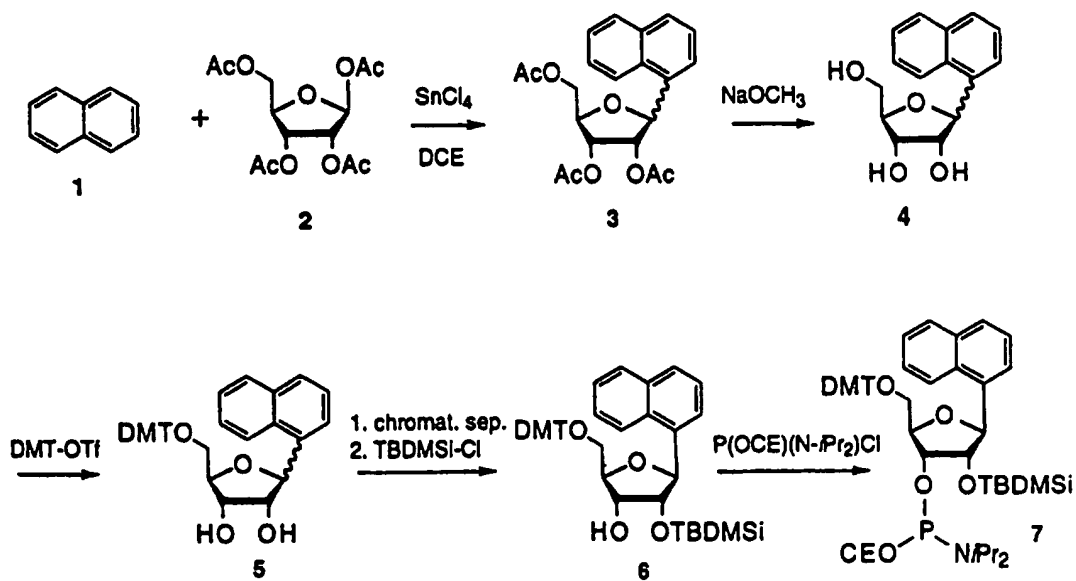
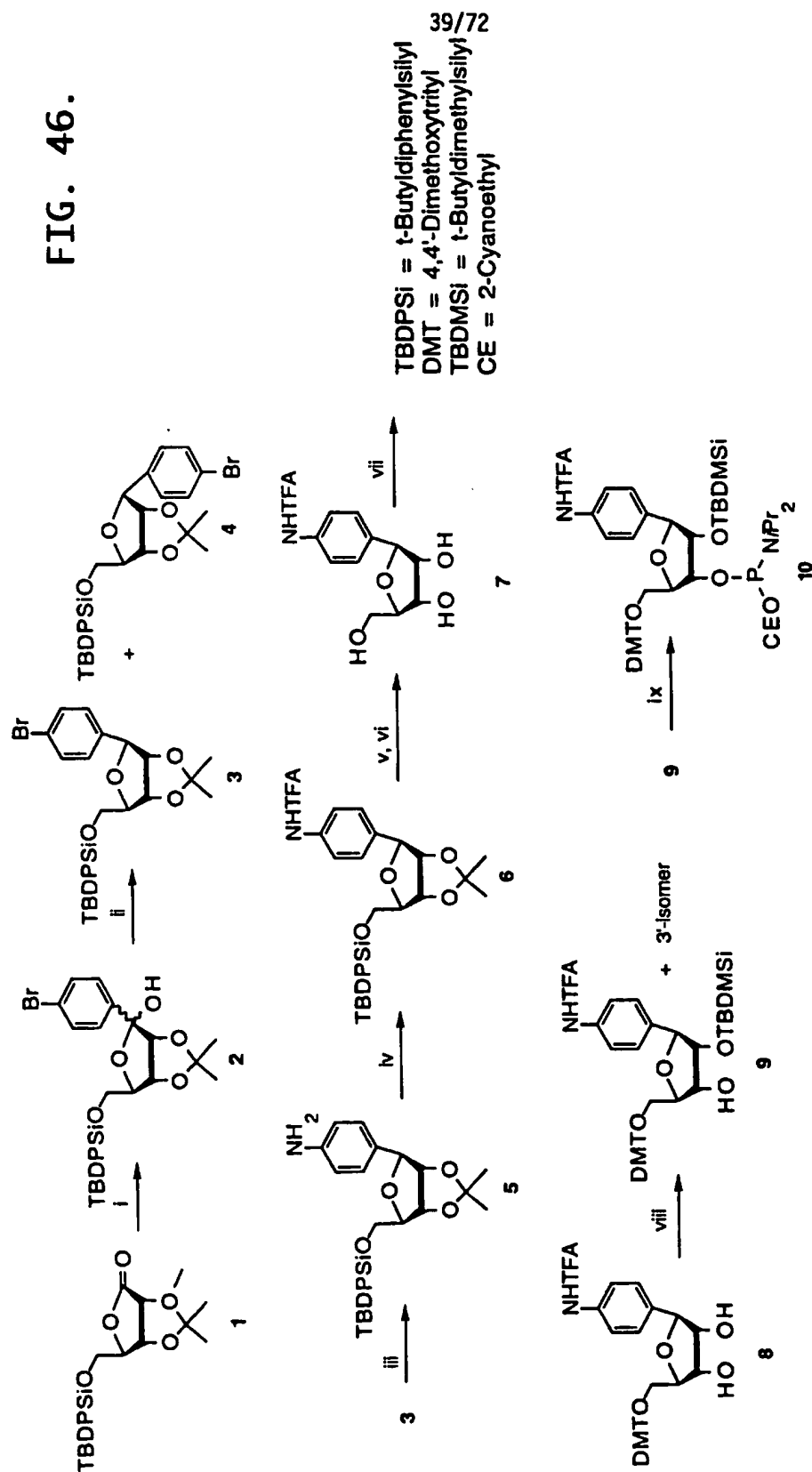


FIG. 46.



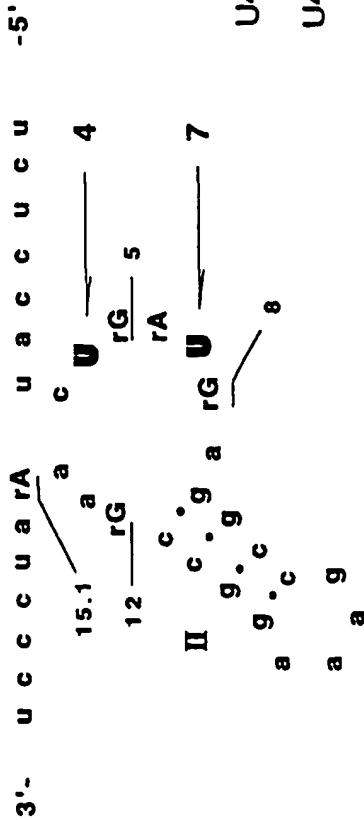


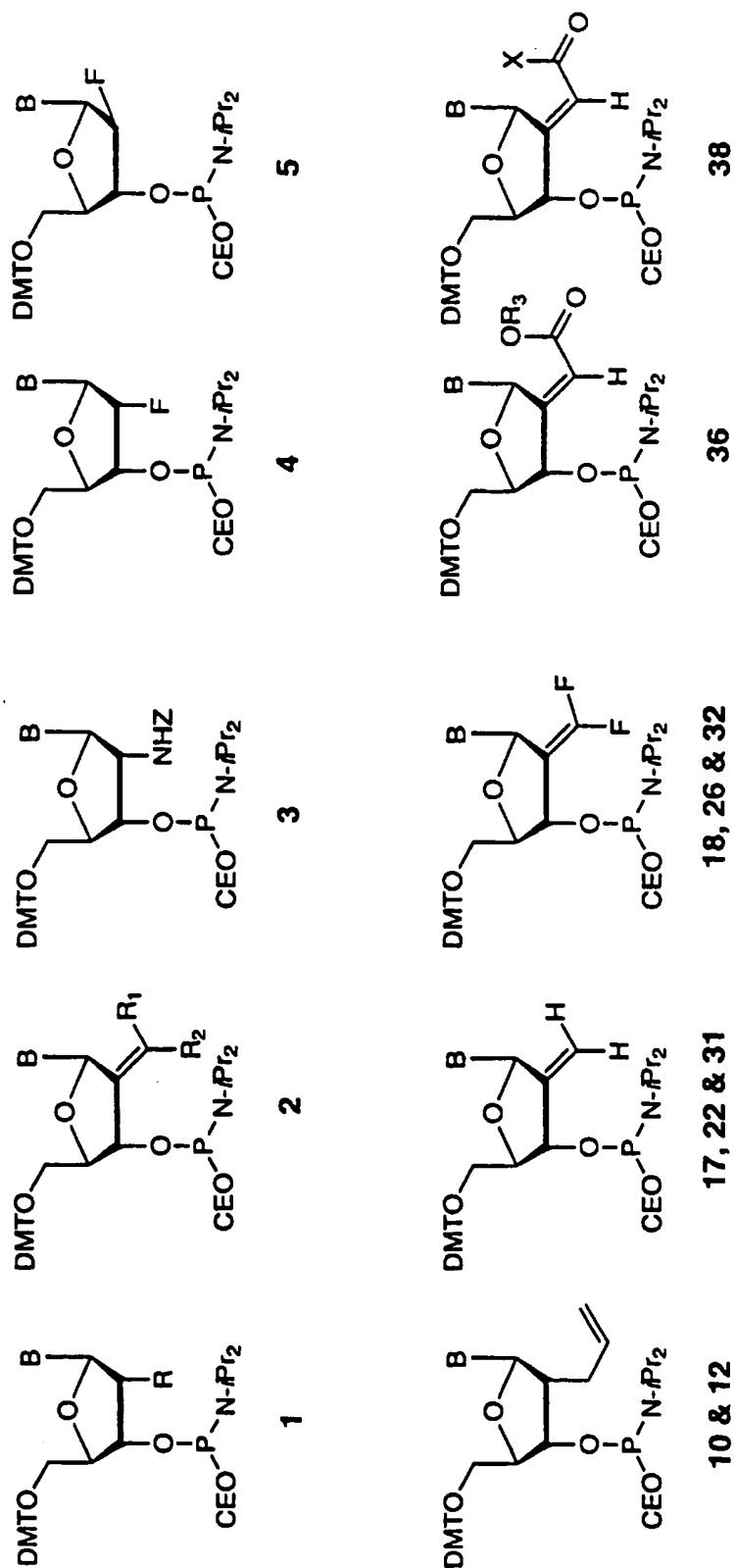
Table 1 Entries

U4 & U7 = 2'-C-Allyl-U	12-14
U4 & U7 = 2'-F-ribo-U	9-11
U4 & U7 = 2'=CH ₂ -U	3-5
U4 & U7 = 2'=CF ₂ -U	6-8
U4 & U7 = 2'-dU	21-22
U4 & U7 = 2'-F-ara-U	15-17
U4 & U7 = 2'-NH ₂ -U	18-20
U4 & U7 = 2'-O-Me-ribo-U	2

Lower case = 2'-O-Me
rN = ribonucleotide

FIG. 47.

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SUBSTITUTE SHEET (RULE 26)

B = Protected A, C, G, U, T, 2AP, I, DiAP, P etc.

FIG. 48.

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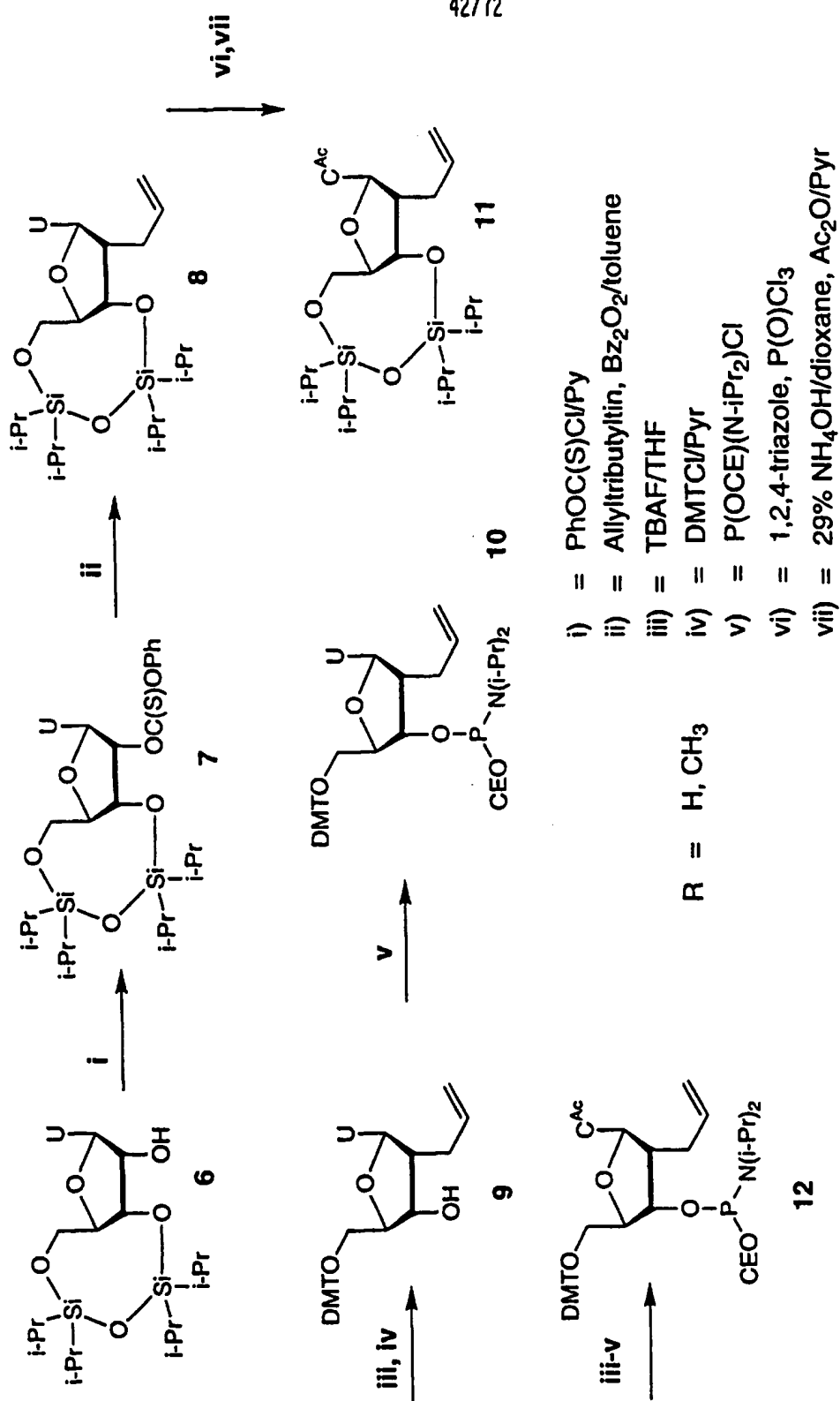
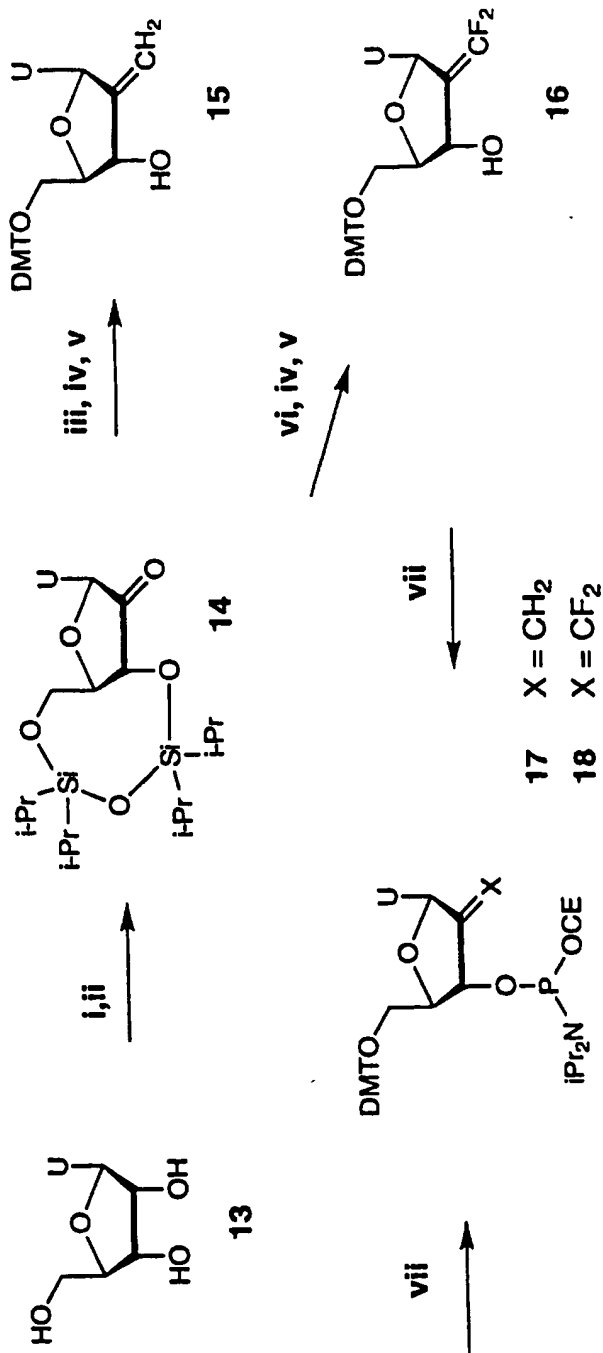


FIG. 49.

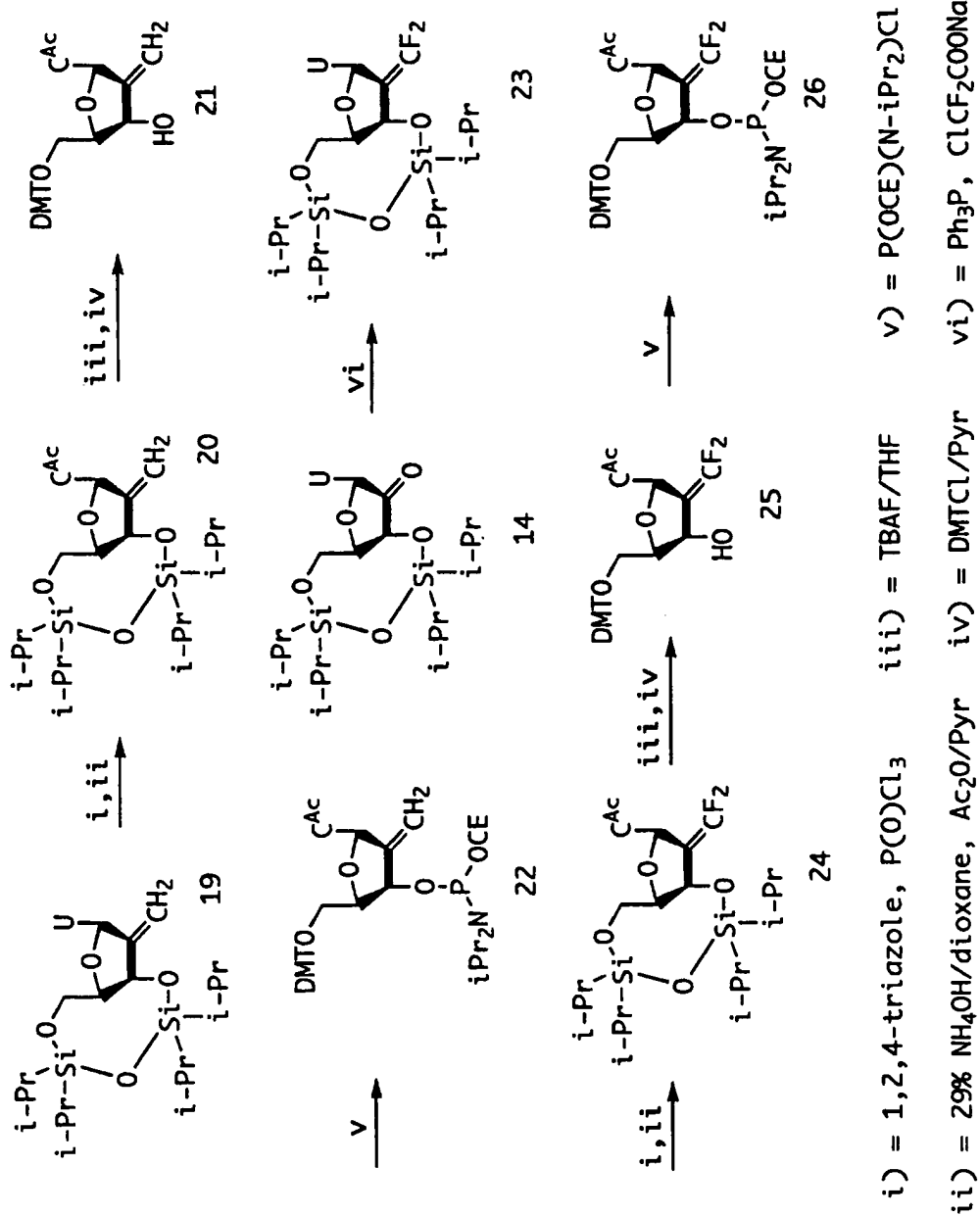
43/72



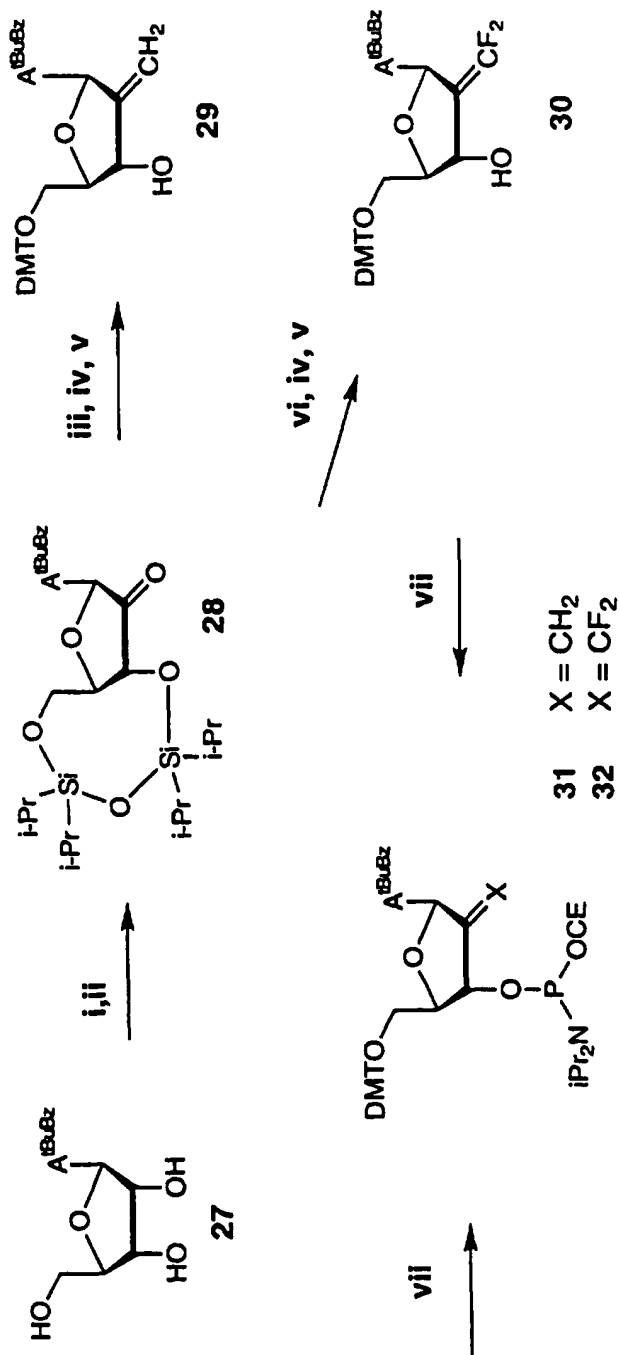
- i) = Markiewicz reagent
 ii) = DMSO & Ac₂O
 iii) = Ph₃PCH₃I
 iv) = TBAF/THF
 v) = DMTCl/Pyr
 vi) = Ph₃P, ClCF₂COONa
 vii) = P(OCE)(N-iPr₂)Cl

FIG. 50.

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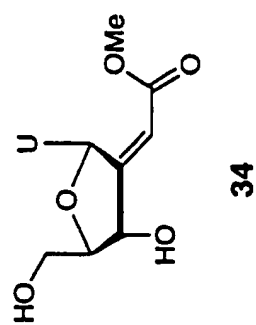
45/72



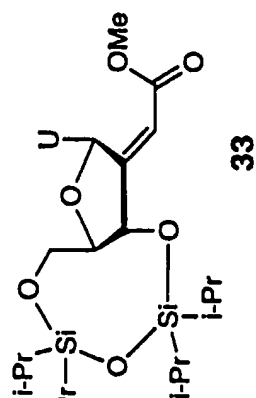
- i) = Markiewicz reagent
 ii) = DMSO & Ac₂O
 iii) = Ph₃PCH₃I
 iv) = TBAF/THF
 v) = DMTCI/Pyr
 vi) = Ph₃P, ClCF₂COONa
 vii) = P(OCE)(N-iPr₂)Cl

FIG. 52.

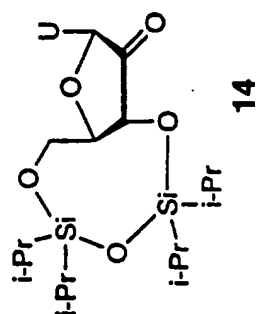
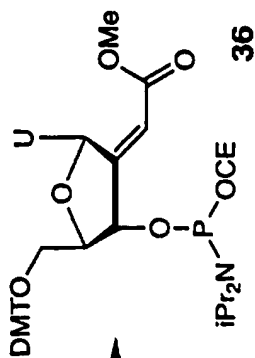
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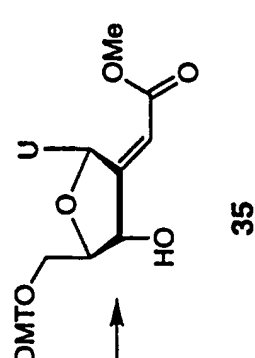
ii



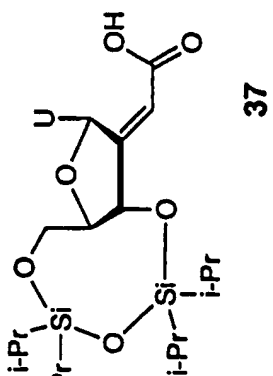
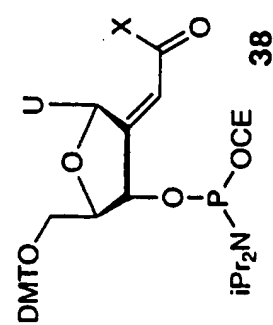
i

i) = $\text{Ph}_3\text{PC}=\text{CHC}(\text{O})\text{OCH}_3\cdot\text{OAc}$ ii) = $\text{NEt}_3\cdot 3 \text{ HF}$ iii) = DMTCI/Pyr iv) = $\text{P}(\text{OCE})(\text{N-iPr}_2)\text{Cl}$ v) = MeOH/NaOH 

iv



iii

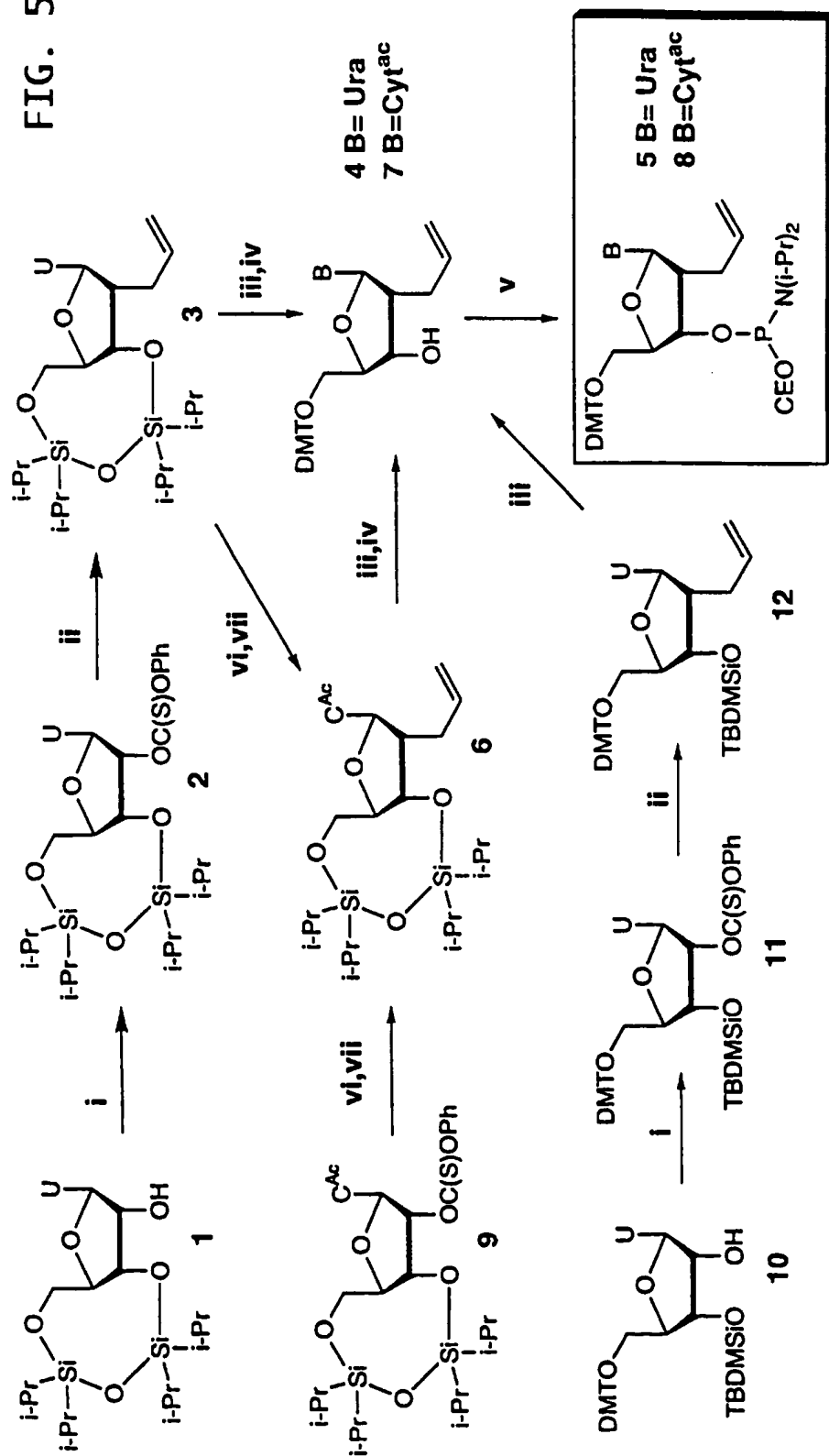


v

33

FIG. 53.

FIG. 54.



i) = PhOC(SiCl₃/Pyr ii) = Allyl(tributyltin, Bz₂O₂ or AIBN /toluene)iii) = TBAF iv) = DMTCI/Pyr
v) = P(OCE)(N-iPr)₂Cl vi) = 1,2,4-triazole, P(O)Cl₃ vii) = 29% NH₄OH/dioxane, Ac₂O/Pyr

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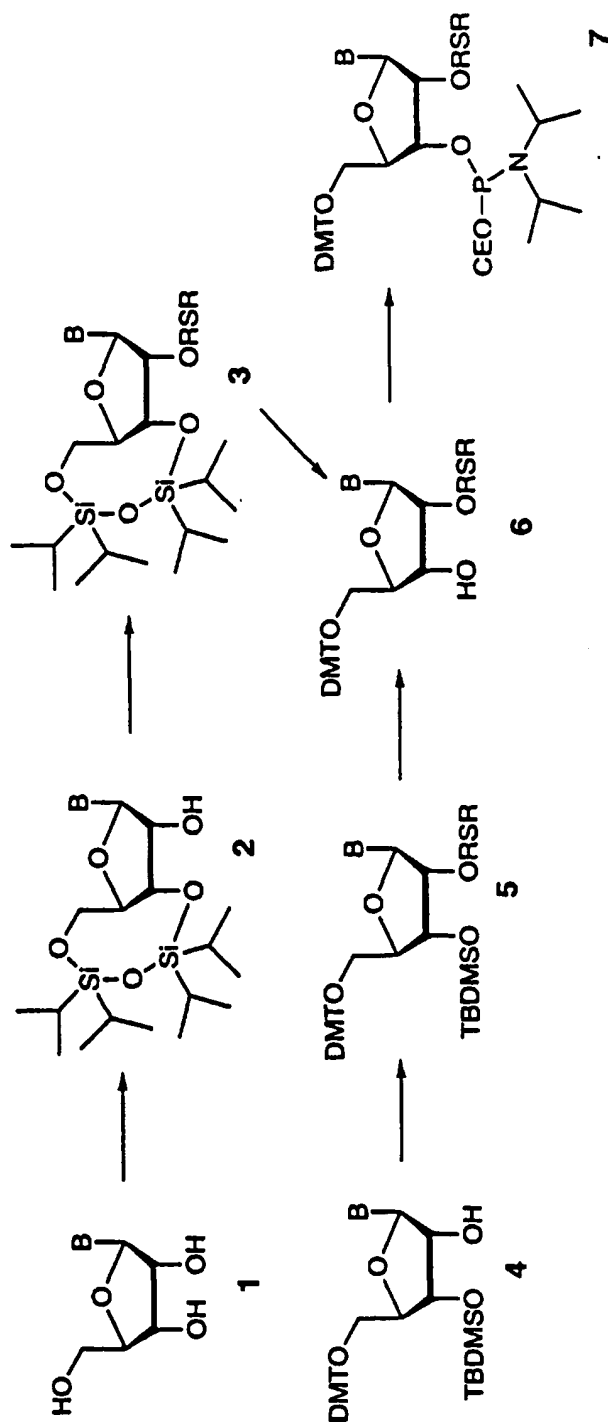


FIG. 55.

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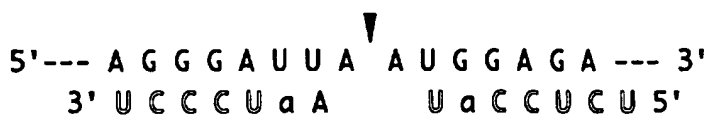
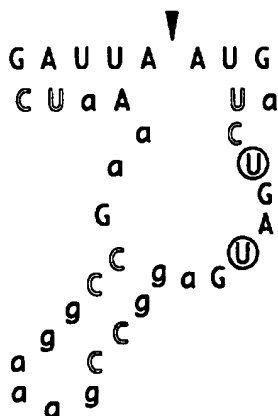


FIG. 56a.



UPPER CASE=ribonucleotides
 LOWER CASE=2'-O-Methyl nucleotides
 U and C=2'-O-Methylthiomethyl
 Ⓢ=2'-Amino

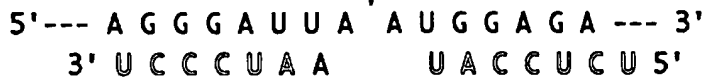
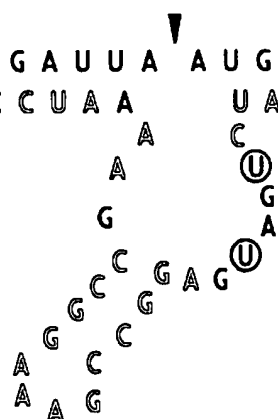


FIG. 56b.



UPPER CASE=ribonucleotides
 LOWER CASE=2'-O-Methyl nucleotides
 U, A, G and C=2'-O-Methylthiomethyl
 Ⓢ=2'Amino

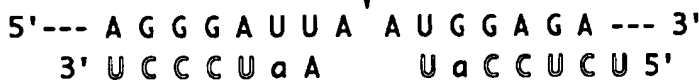
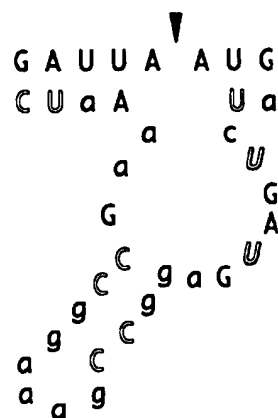


FIG. 56c.



UPPER CASE=ribonucleotides
 LOWER CASE=2'-O-Methyl nucleotides
 C=2'-O-Methylthiomethyl
 Ⓢ=2'Amino

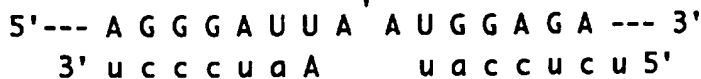
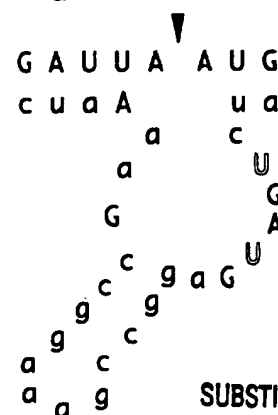


FIG. 56d.



UPPER CASE=ribonucleotides
 LOWER CASE=2'-O-Methyl nucleotides
 U=2'-Methylthiomethyl

SUBSTITUTE SHEET (RULE 26)

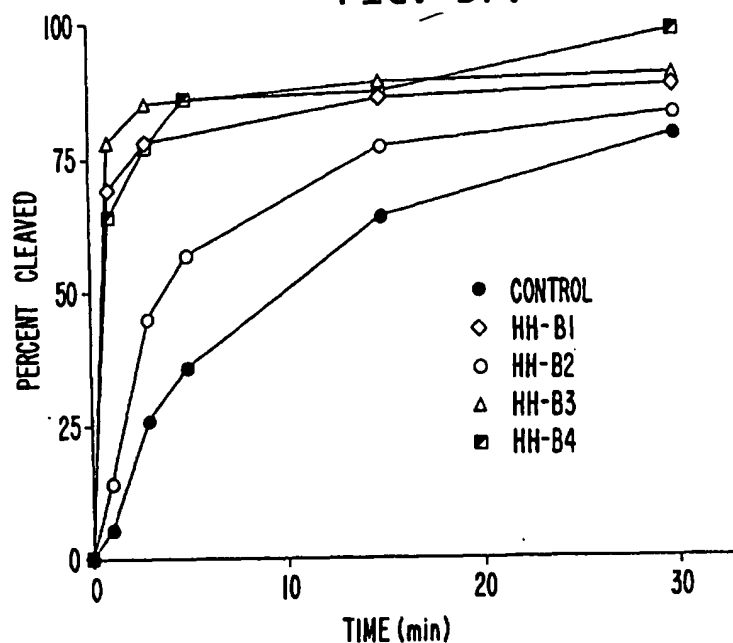
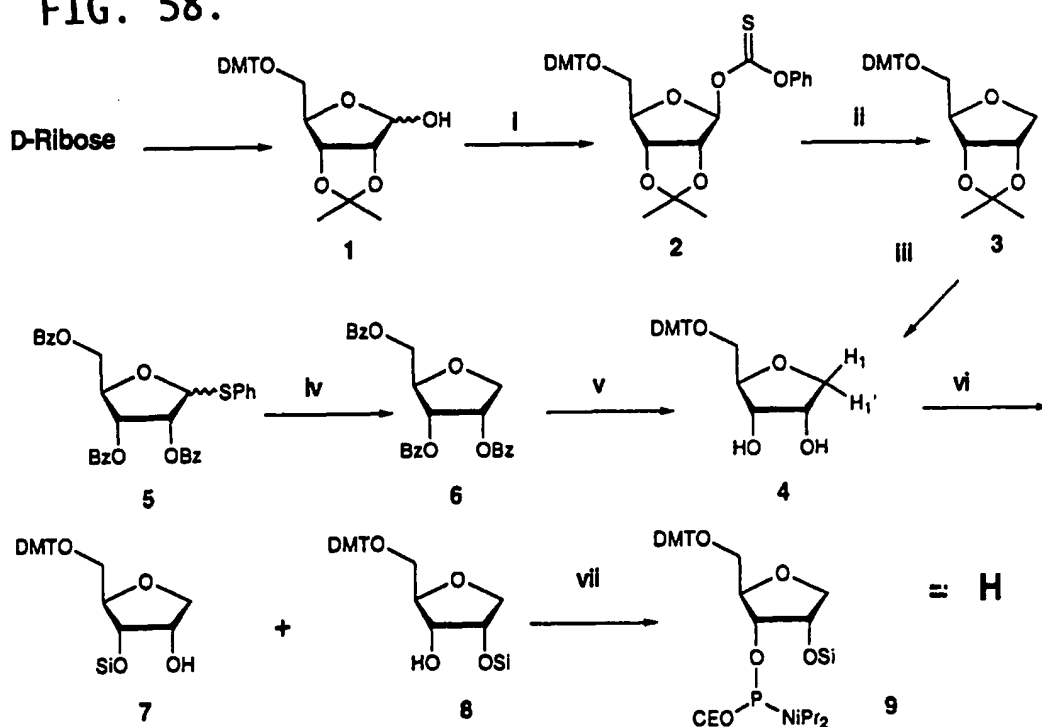
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FIG. 57.

FIG. 58.



Si = *t*-Butyldimethylsilyl
 DMT = 4,4'-Dimethoxytrityl
 CE = Cyanoethyl

Reagents and Conditions: i) PhOC(S)-Cl/DMAP, ii) Bu₃SnH/AIBN, iii) CF₃COOH, DMT-Cl/Pyr, iv) Bu₃SnH/Bz₂O₂, v) 2M NaOH/Pyr/MeOH, DMT-Cl/Pyr, vi) TBDMS-Cl/AgNO₃, vii) P(OCE)(N-*i*Pr₂)Cl

SUBSTITUTE SHEET (RULE 26)

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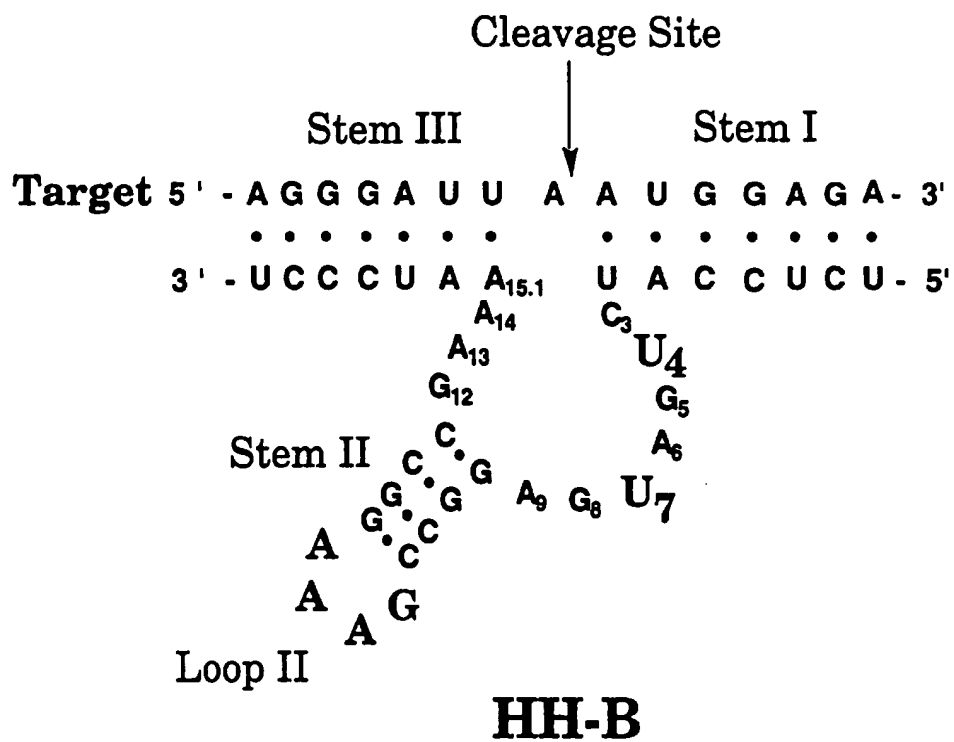


FIG. 59.

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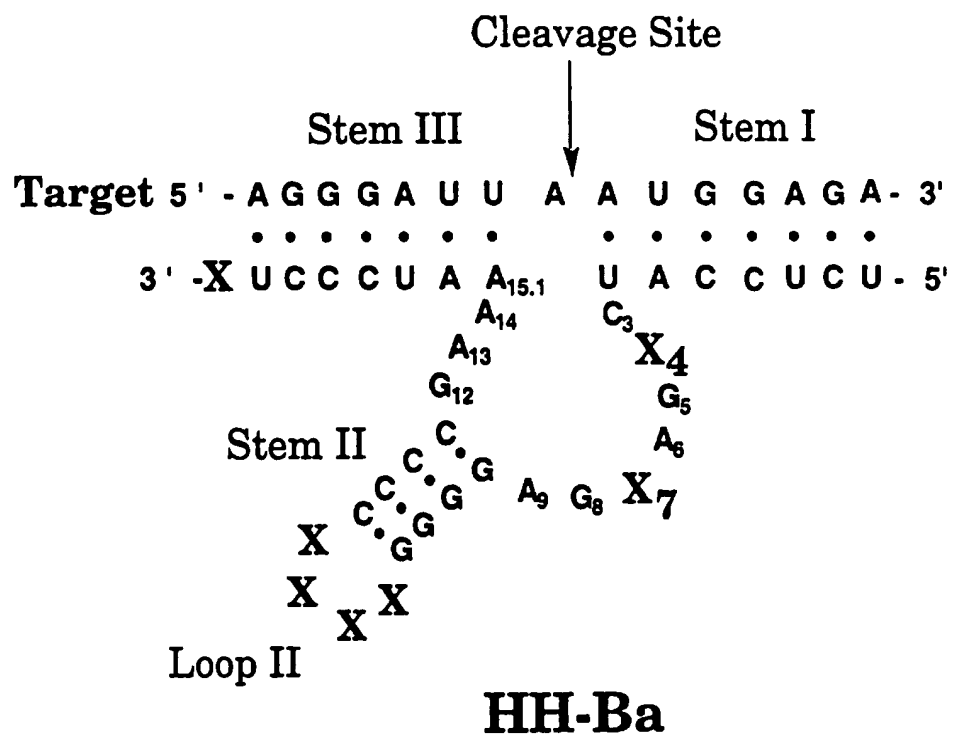


FIG. 60.

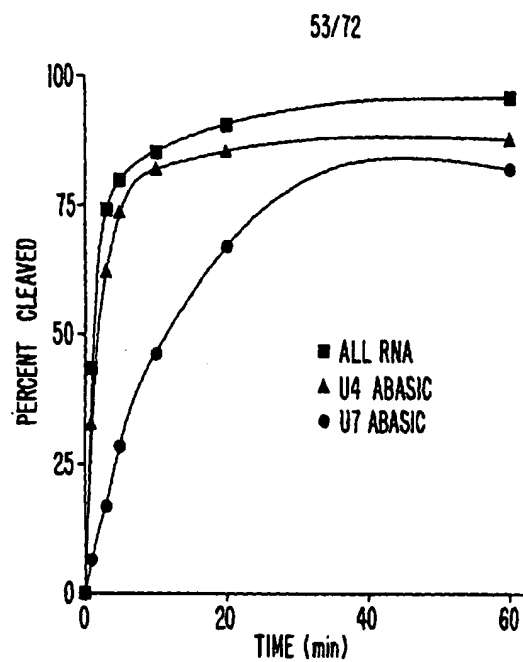


FIG. 61.

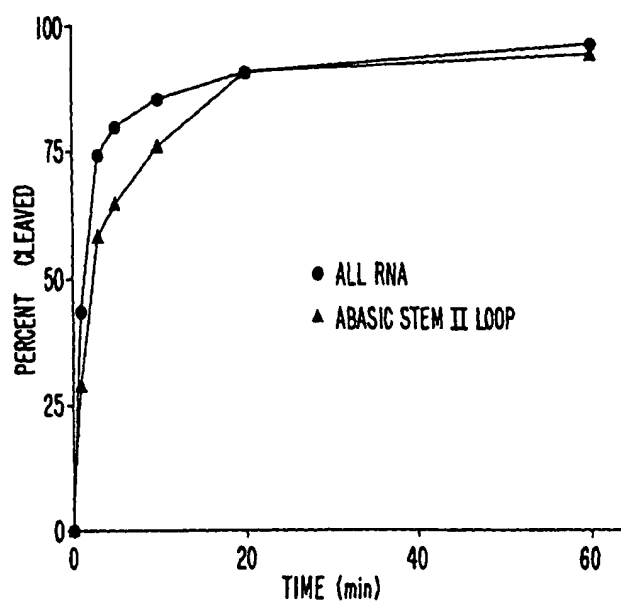


FIG. 62.

SUBSTITUTE SHEET (RULE 26)

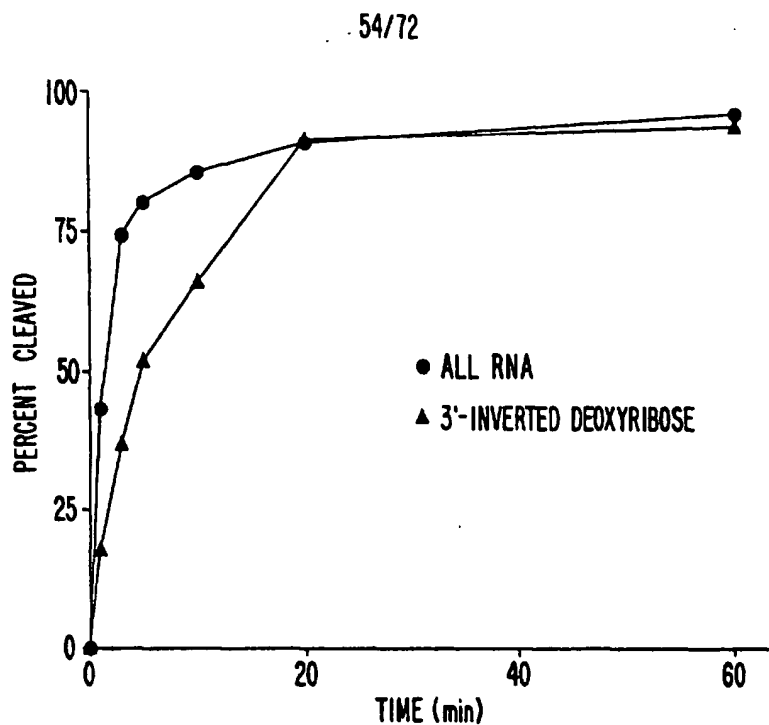


FIG. 63.

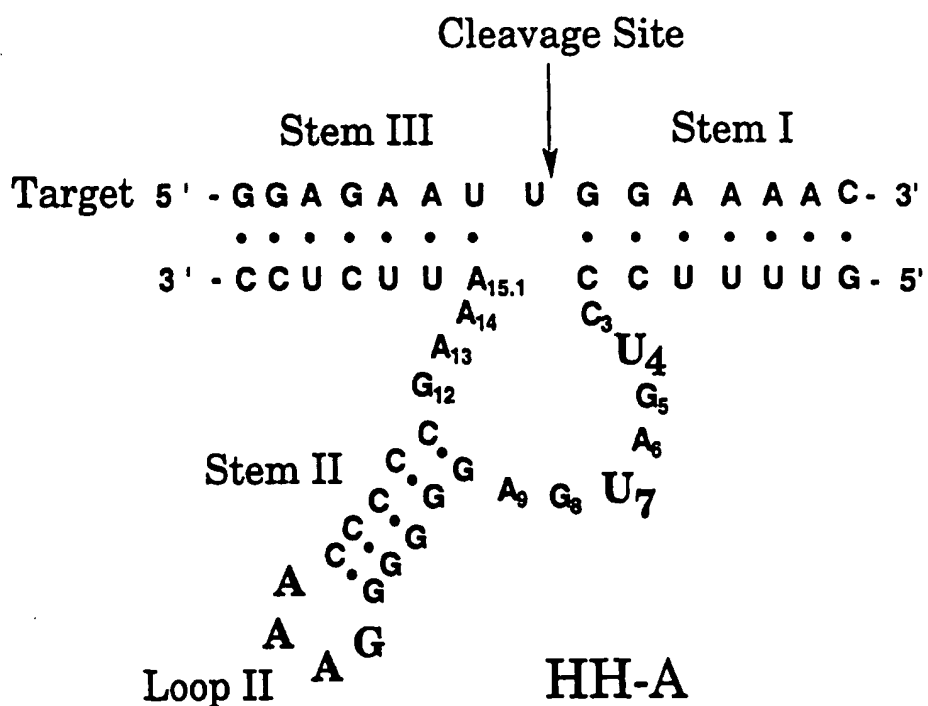


FIG. 64.

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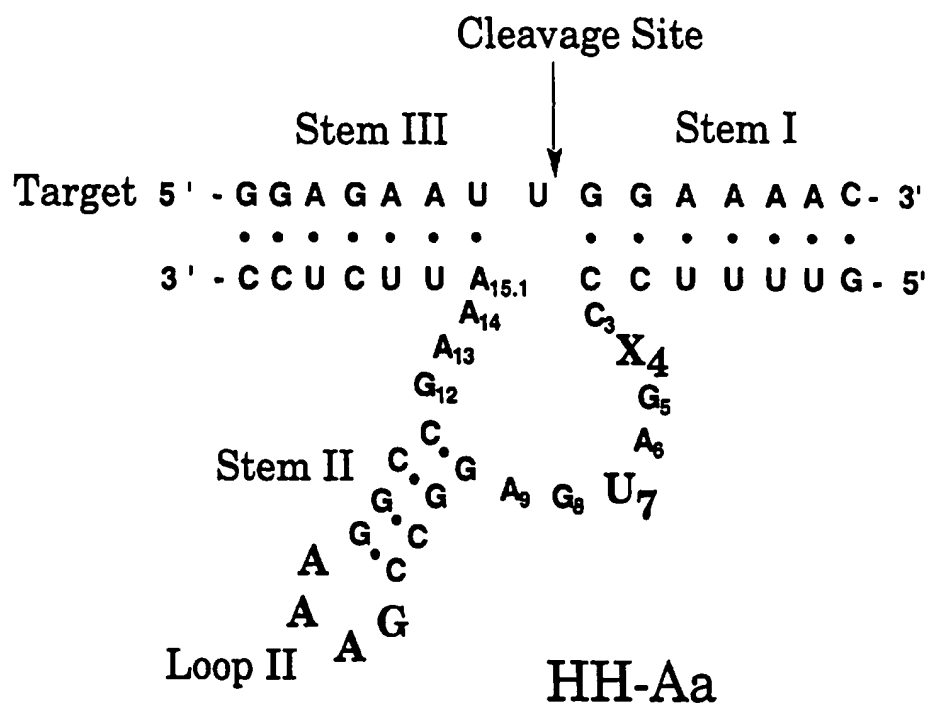


FIG. 65.

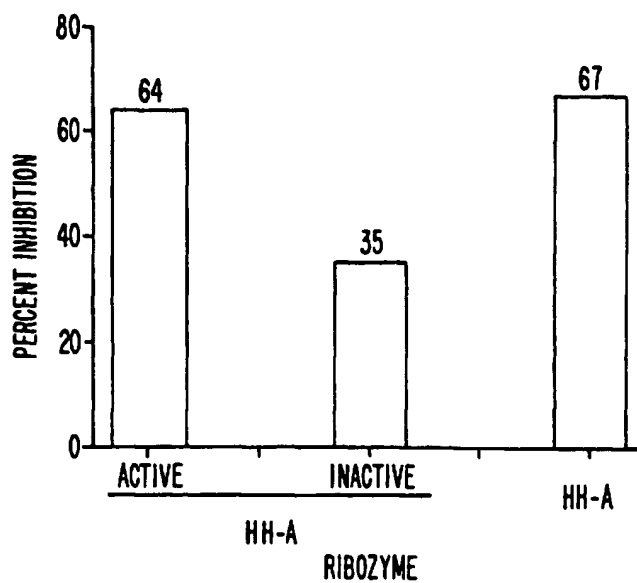
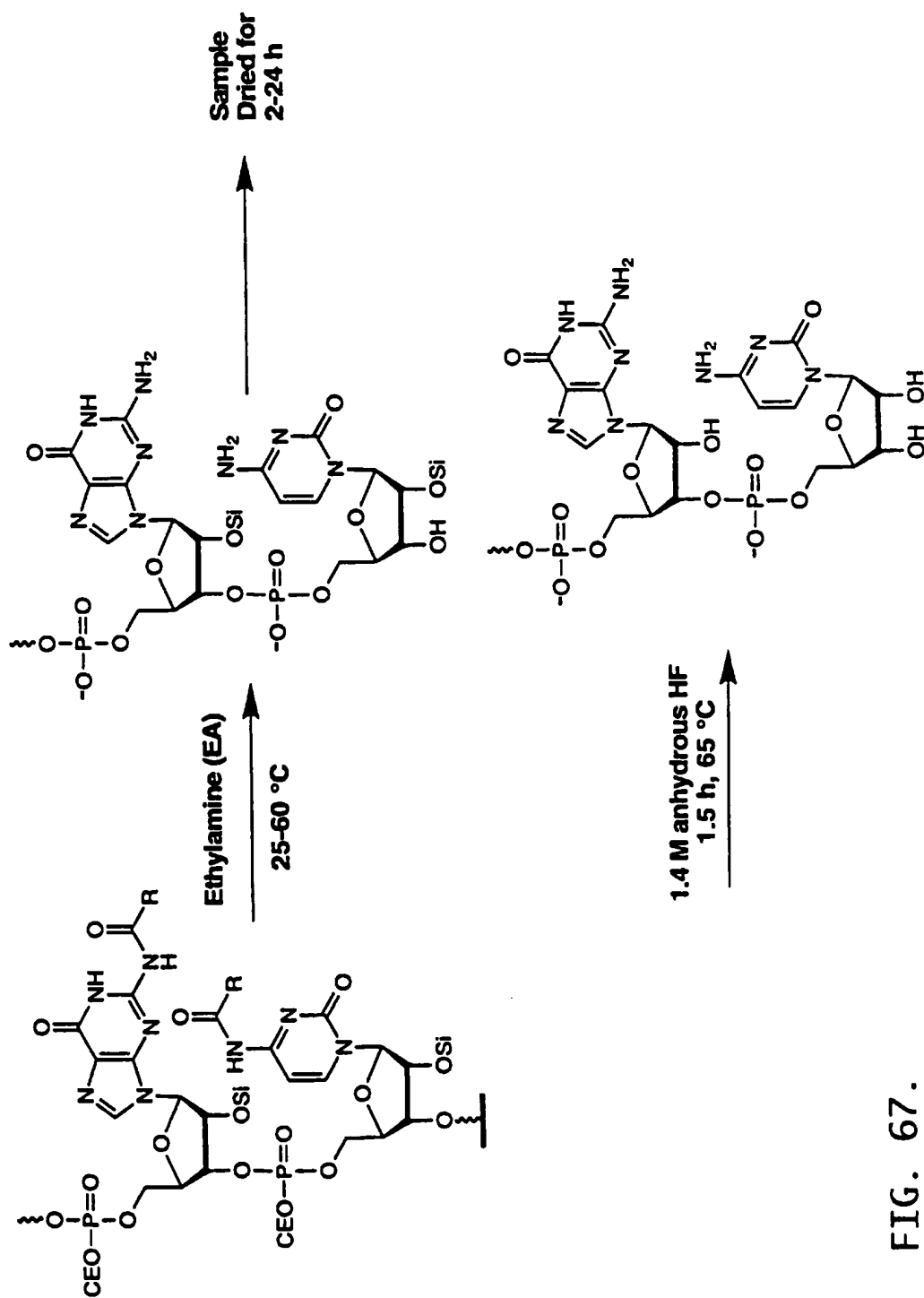


FIG. 66.
SUBSTITUTE SHEET (RULE 26)

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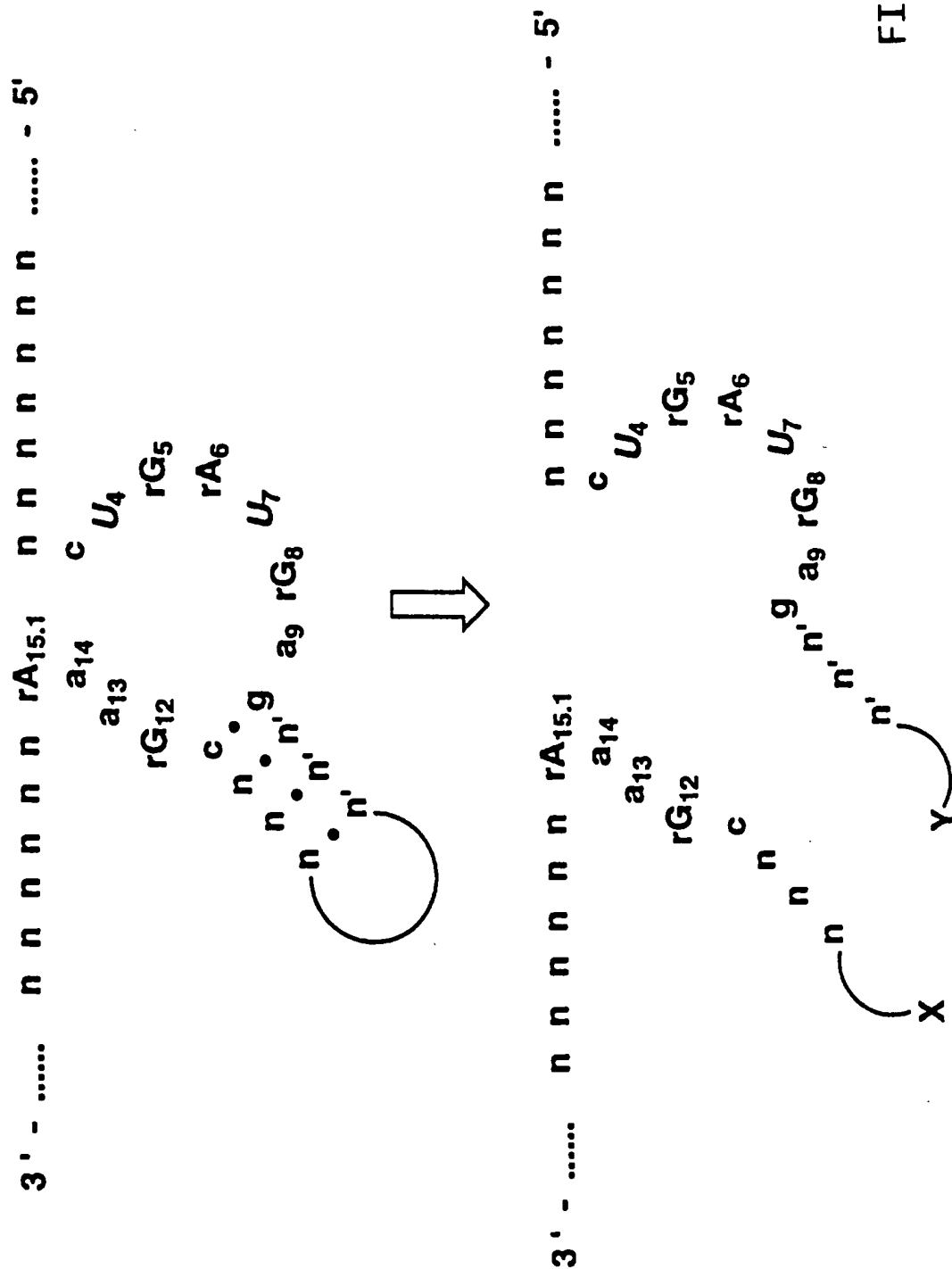
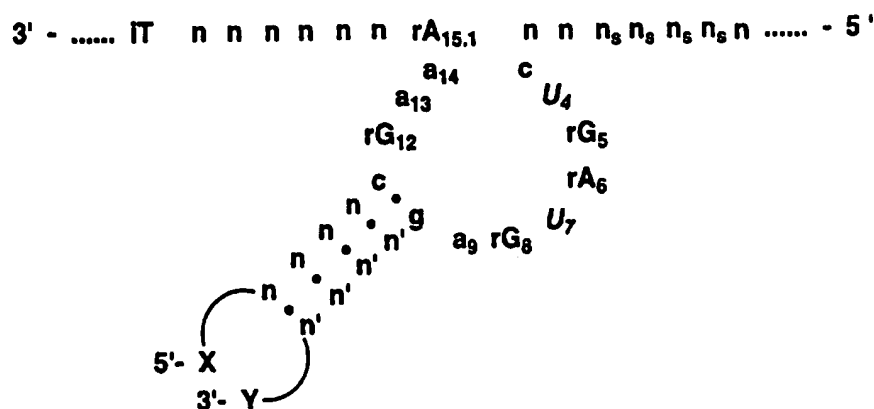


FIG. 68.

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NOTE: $(CH_2)_n$ refers to any linkage. In addition, X and Y can be interchanged.

$$X = (\text{CH}_2)_n\text{SH}, Y = (\text{CH}_2)_n\text{SH} \rightarrow \text{disulfide}$$

X = (CH₂)_nNHR, Y = ribose → morpholino

$$X = (\text{CH}_2)_n\text{NHR}, Y = \text{CO}_2\text{H} \longrightarrow \text{amide}$$
$$X = (\text{CH}_2)_n\text{X}, \quad Y = (\text{CH}_2)_n\text{OH} \rightarrow \text{ether, X = halogen}$$
$$X = (\text{CH}_2)_n\text{NHR}, Y = \text{CHO} \longrightarrow \text{amine}$$

$X = (CH_2)_n PPh_3, Y = CHO \longrightarrow$ double bond

$X = (CH_2)_nNHR$, $Y = (CH_2)_nSO_2Cl$ → sulfonamide

$$X = (\text{CH}_2)_n\text{OH}, Y = \text{CO}_2\text{H} \quad \longrightarrow \quad \text{ester}$$
$$X = (\text{CH}_2)_n\text{X}, \quad Y = (\text{CH}_2)_n\text{SH} \longrightarrow \text{thioether, } X = \text{halogen}$$
$$X = (\text{CH}_2)_n\text{COX}, \quad Y = (\text{CH}_2)_n\text{OH} \rightarrow \text{carbonate, } X = \text{halogen}$$

FIG. 69.

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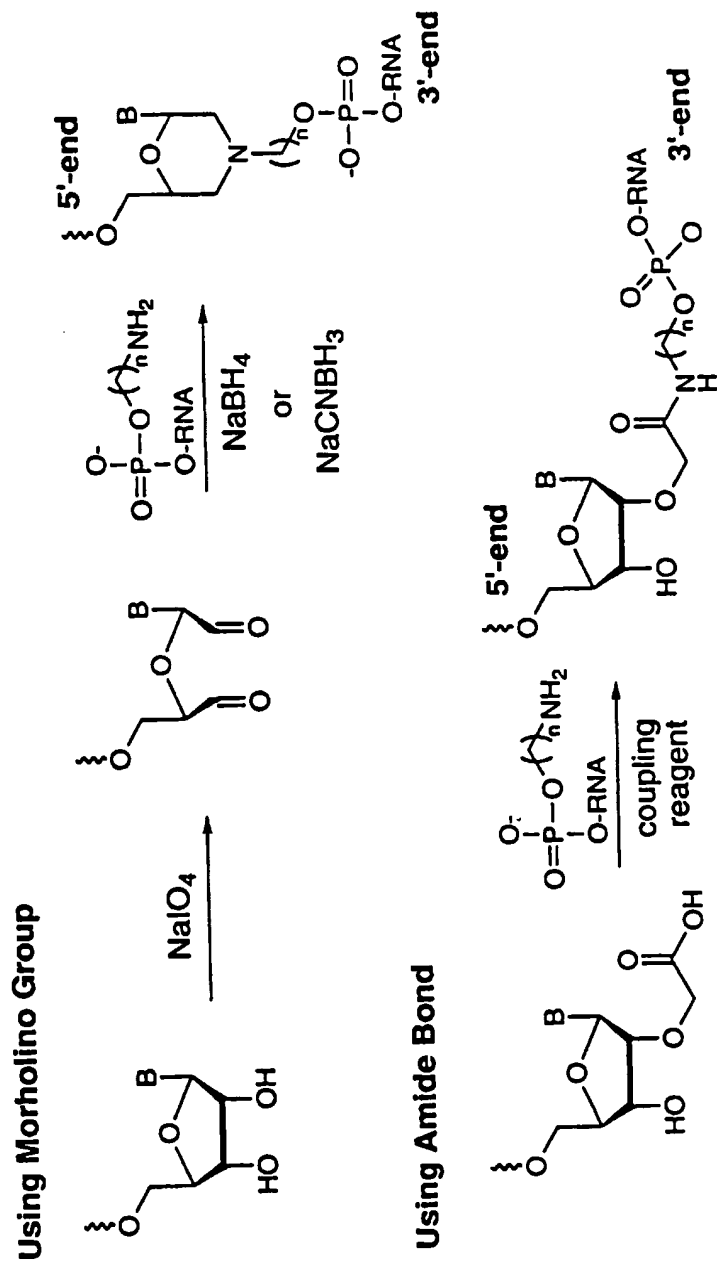
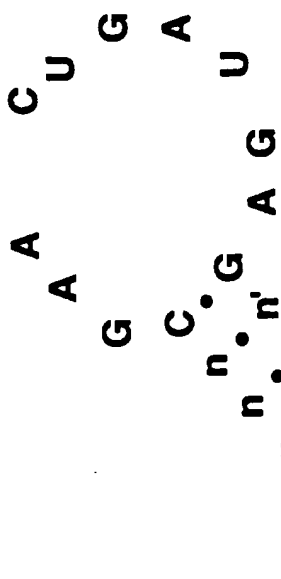


FIG. 70.

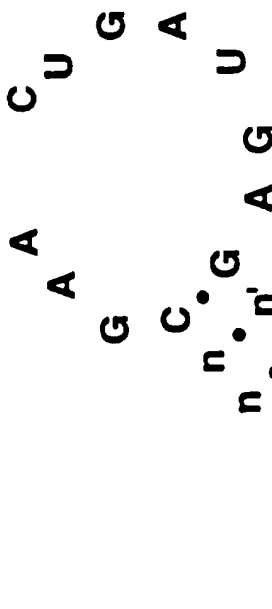
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3' - CCUCUUA CCUUUUG - 5'



HH-A1

3' - CCUCUUA CCUUUUG - 5'



HH-A2; x = 5
HH-A3; x = 6

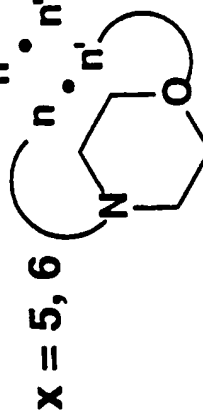


FIG. 71.

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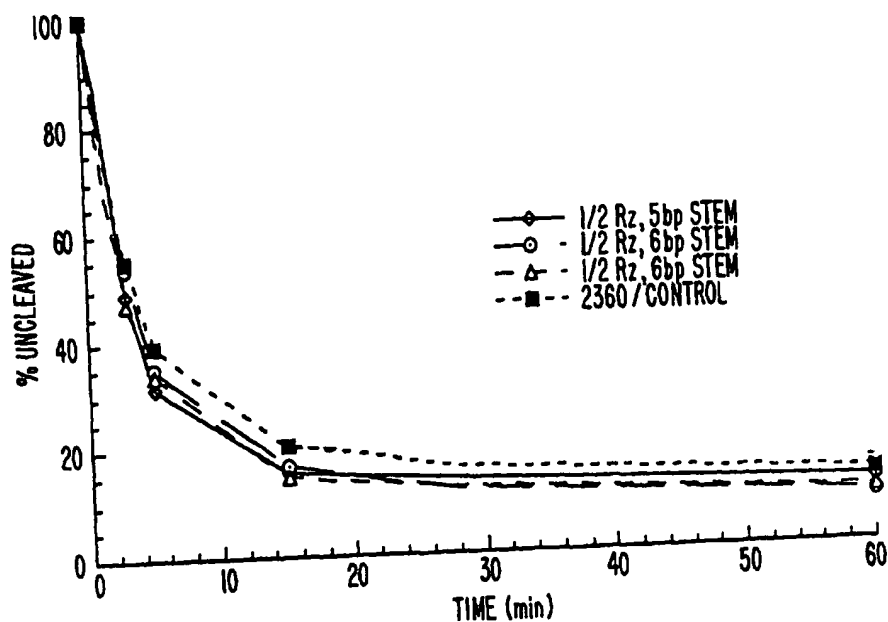


FIG. 72.

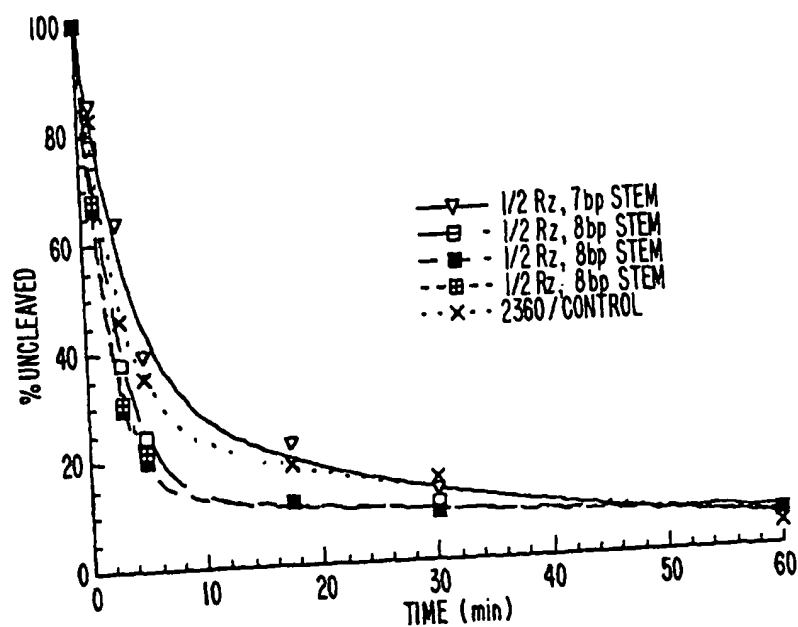


FIG. 73.

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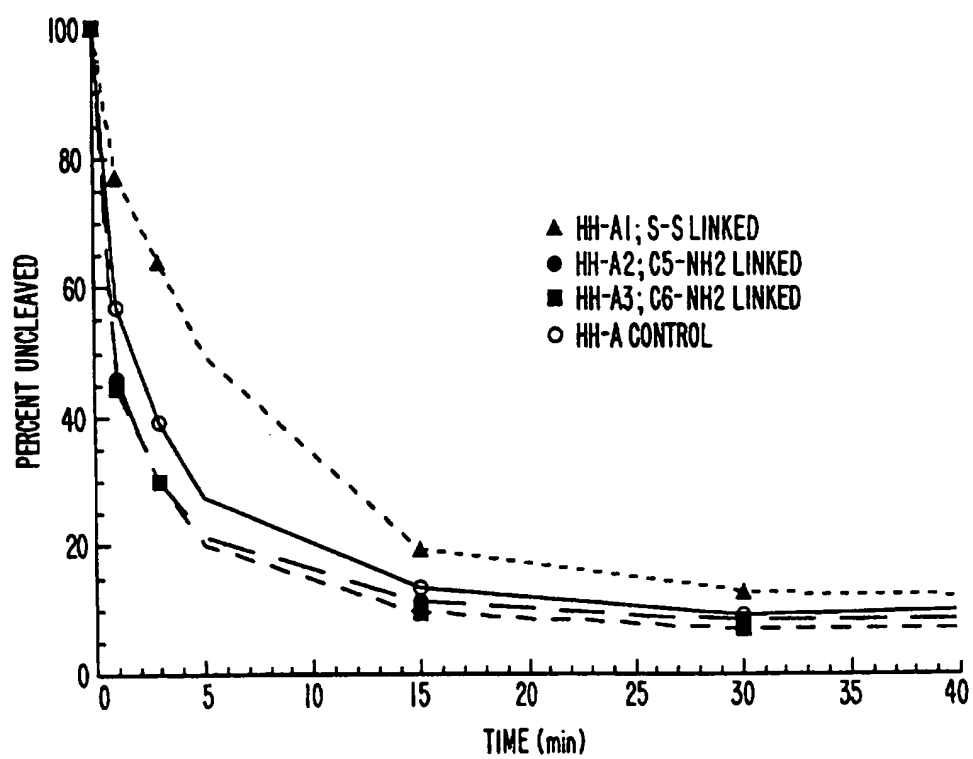
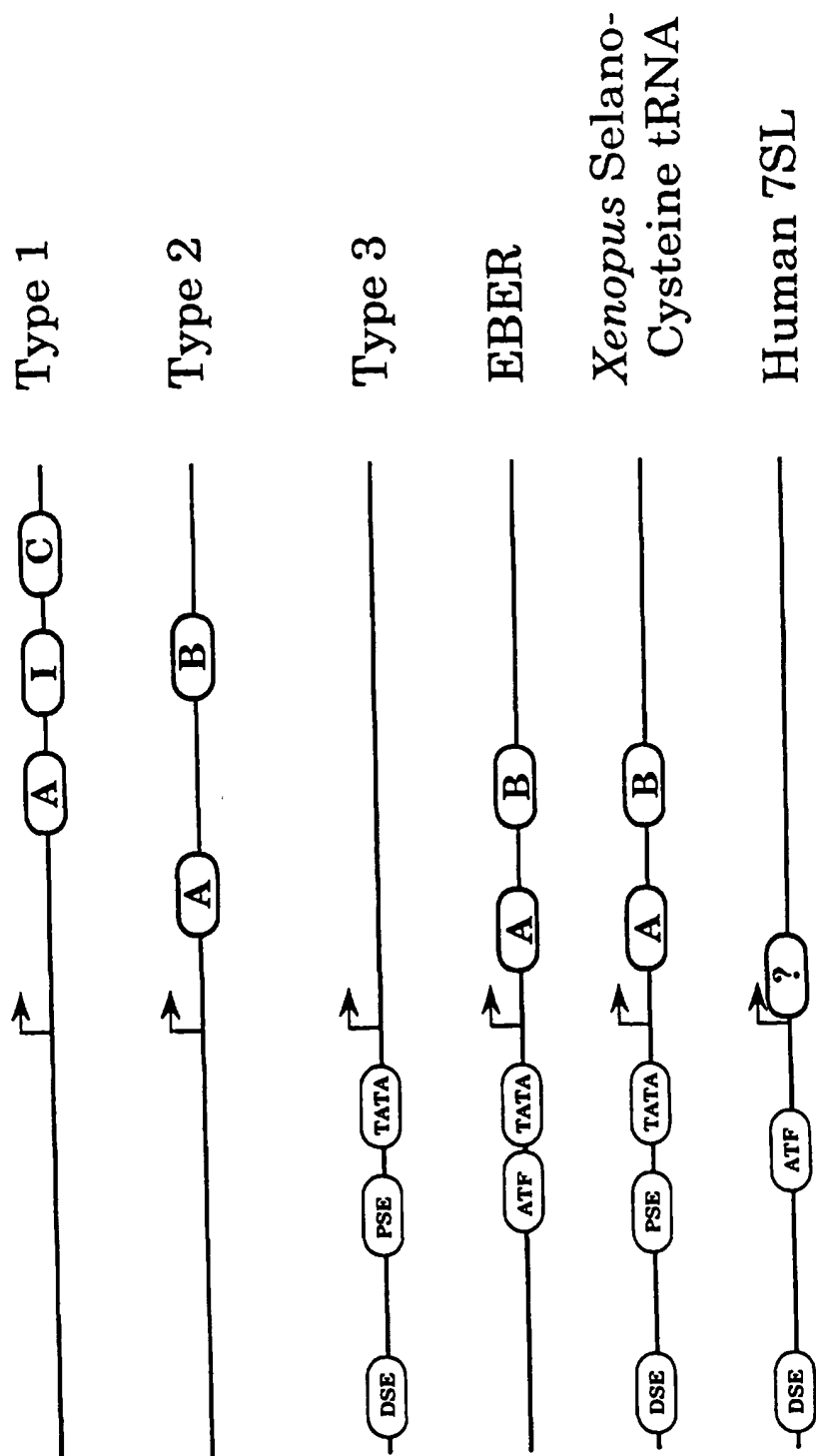


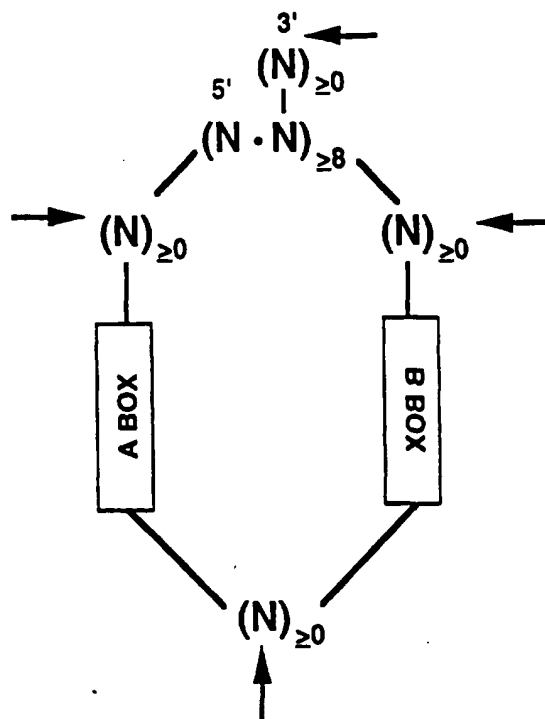
FIG. 74.

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FIG. 75.



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A BOX = URGCNNAGYGG

B BOX = GGUUCGANUCC

This is based on Geiduschek & Tocchini-Valentini, (1988) *Annu. Review Biochem.* 57, 873-914. However this consensus sequence is not meant to be limiting

N = A, U, G, or C

R = Purine

Y = Pyrimidine

• = Indicates base-pairing

— = Indicates covalent linkage

➔ = Indicates sites at which desired RNAs can be cloned

FIG. 76.

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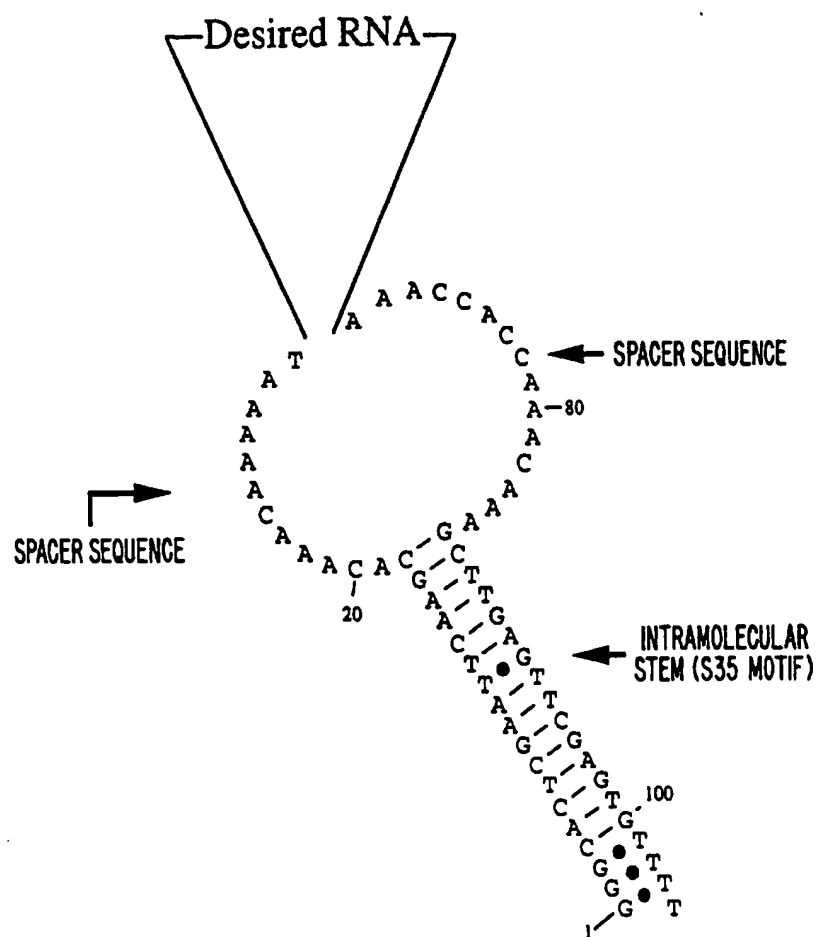
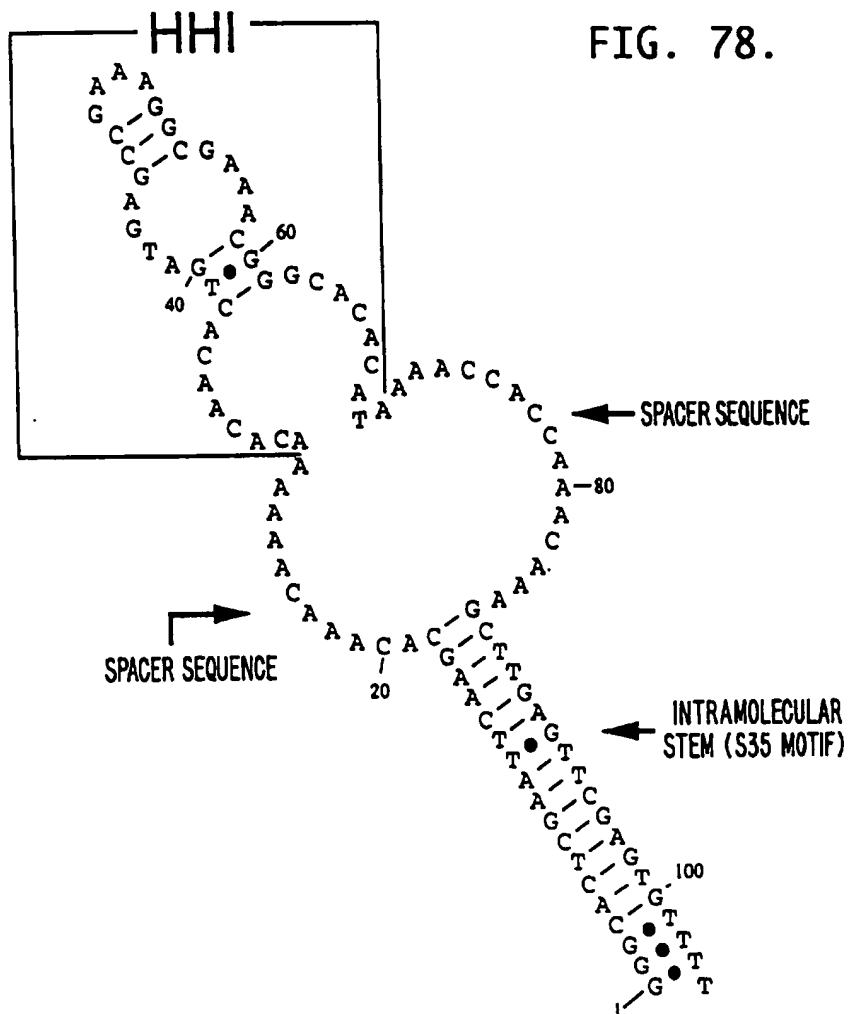


FIG. 77.

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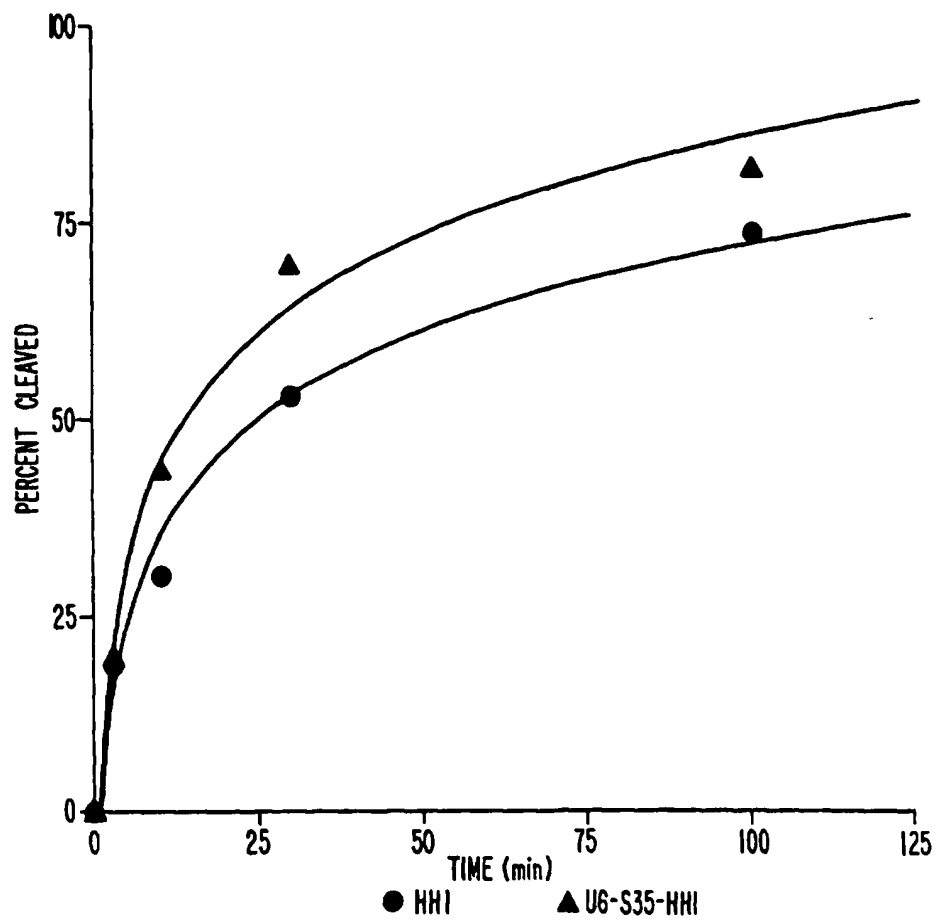


FIG. 80.

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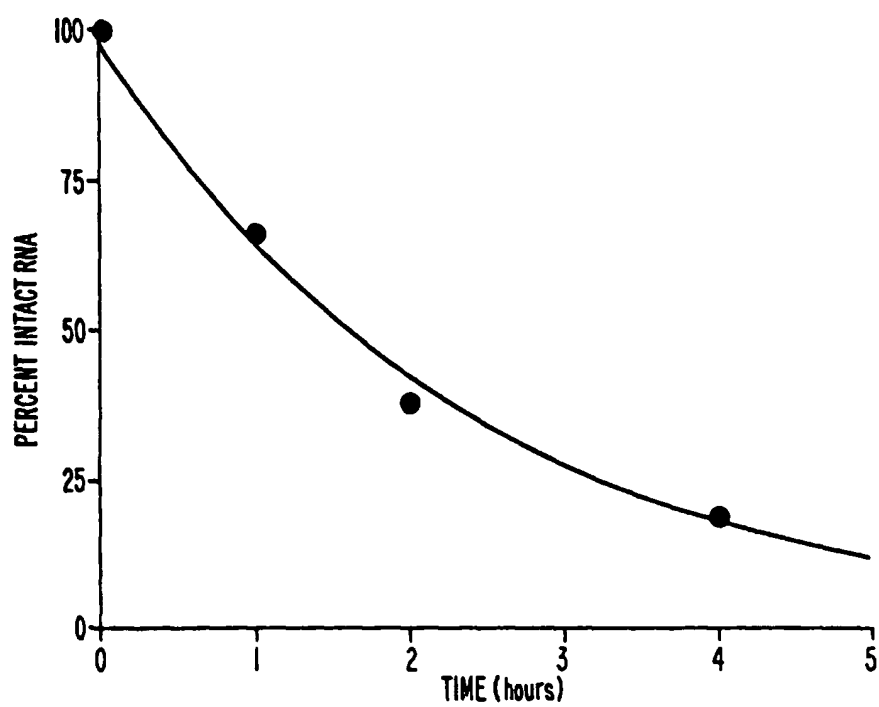
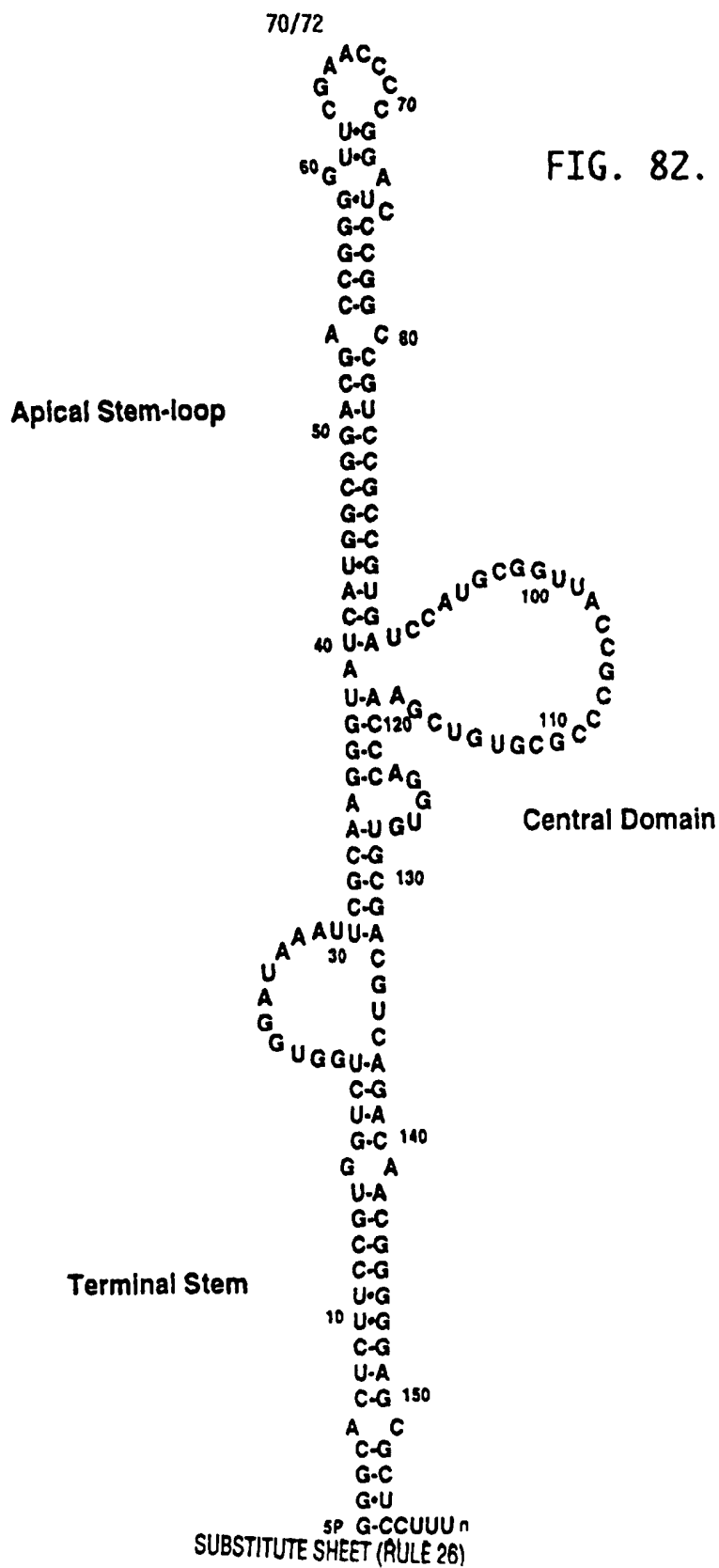
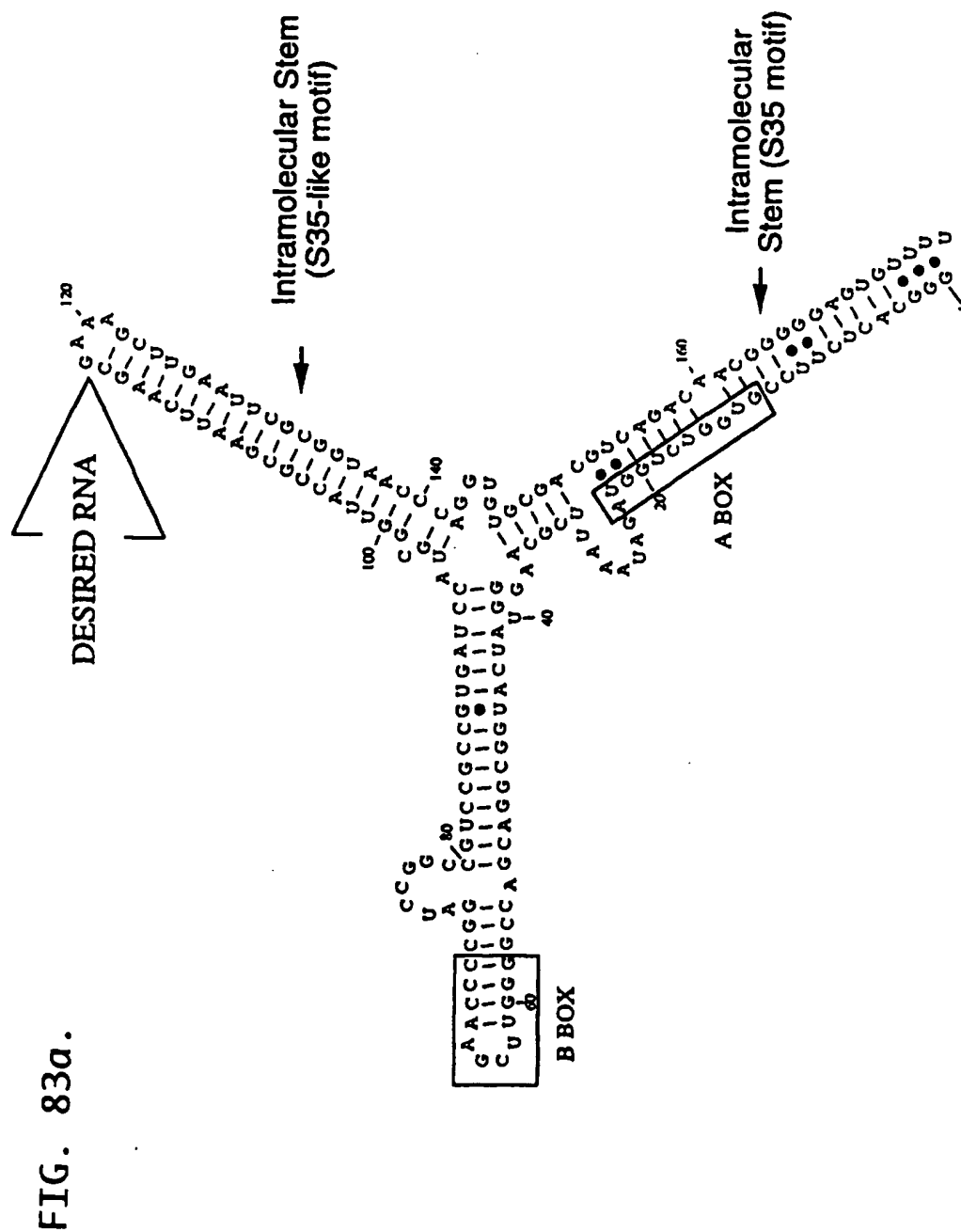
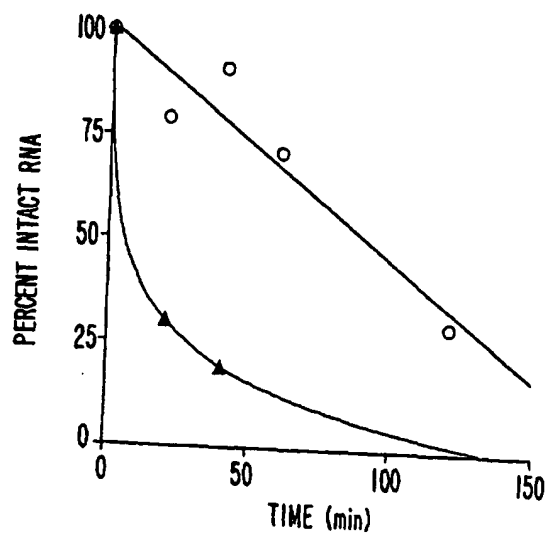
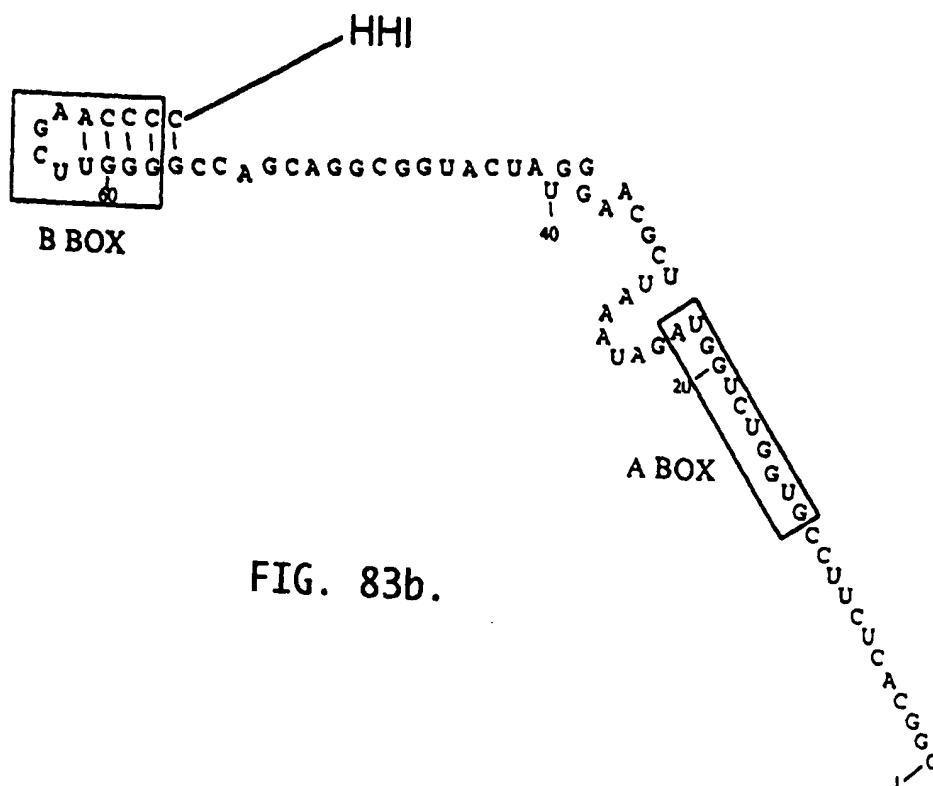


FIG. 81.





VA1-Chimera



▲ VAI-CHIMERA
○ VAI-S35-CHIMERA

FIG. 84.
SUBSTITUTE SHEET (RULE 26)

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